PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C07K 15/00, C12P 21/02
C12N 15/24, 15/27, 15/70
C12N 5/00, 5/10, A61K 37/02
C07H 15/12

A1

(11) International Publication Number: WO 92/06116

(43) International Publication Date: 16 April 1992 (16.04.92)

(21) International Application Number: PCT/US91/07053

(22) International Filing Date: 26 September 1991 (26.09.91)

(30) Priority data:
589,958 28 September 1990 (28.09.90) US

(71) Applicant: ORTHO PHARMACEUTICAL CORPORATION [US/US]; Route 202, Raritan, NJ 08869-0602 (US).

(72) Inventor: ROSEN, Jonathan, I. ; 10264 Meadowview Drive, San Diego, CA 92131 (US).

(74) Agent: MINIER, Robert, L.; Johnson & Johnson, One Johnson & Johnson Plaza, New Brunswick, NJ 08933-7003 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: HYBRID GROWTH FACTORS

(57) Abstract

The present invention provides recombinant hematopoietic molecules comprising at least a portion of a first hematopoietic molecule having early myeloid differentiation activity and at least a portion of a second hematopoietic molecule having late myeloid differentiation activity. Nucleic acid molecules encoding such recombinant molecules, as well as pharmaceutical compositions comprising such recombinant factors are also disclosed.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MN	Mongolia
BE	Belgium	GA	Gabon	MR	Mauritania
BF	Burkina Faso	GB	United Kingdom	MW	Malawi
BG	Bulgaria	GN	Guinea	NL	Netherlands
BJ	Benin	GR	Greece	NO	Norway
BR	Brazil	HU	Hungary	PL	Poland
CA	Canada	IT	Italy	RO	Romania
CF	Central African Republic	JP	Japan	SD	Sudan
CG	Congo	KP	Democratic People's Republic of Korea	SE	Sweden
CH	Switzerland			SN	Senegal
CI	Côte d'Ivoire	KR	Republic of Korea	SU+	Soviet Union
CM	Cameroon	LI	Liechtenstein	TD	Chad
CS	Czechoslovakia	LK	Sri Lanka	TG	Togo
DE*	Germany	LU	Luxembourg	US	United States of America
DK	Denmark	MC	Monaco		

\+ **Any designation of "SU" has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.**

HYBRID GROWTH FACTORS

BACKGROUND OF THE INVENTION

Within this application several publications are referenced by Arabic numerals within parentheses. Full citations for these references may be found at the end of the specification immediately preceding the Sequence Listing. The disclosures of these publications in their entirety are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

A variety of factors can influence the activity of a cell. Frequently a factor exerts its influence by interacting with a receptor on the surface of a cell. After binding to the receptor, the signal which determines the cellular response to the factor can be mediated through a number of different events, including internalization of the factor or alterations of the receptor caused by ligand binding. During the course of hematopoietic differentiation, a number of different factors are involved in the maturation of a pluripotent stem cell into a fully differentiated cell. The activities of these factors during the course of hematopoietic differentiation have resulted in these factors being characterized as early factors or late factors. For example, factors such as interleukin-3 (IL-3) and granulocyte-macrophage colony stimulating factor (GM-CSF) are considered early factors, while erythropoietin (Epo), macrophage colony stimulating factor (M-CSF), and granulocyte colony stimulating factor (G-CSF) are considered late factors.

Based upon studies performed with purified factors and *in vitro* colony forming unit assays, it appears that both IL-3 and GM-CSF act on pluripotent cells before they become committed to a particular hematopoietic pathway. After the events stimulated by these factors are underway, such lineage restricted cells become receptive to further differentiation mediated by such late factors as Epo, (which leads to the maturation of erythrocytes), G-CSF (which leads cells

into the granulocytic pathway), and M-CSF (which leads to the maturation of macrophages). Experiments described in recent publications (1,2,3) have demonstrated in vitro that early or late factors alone are poor stimuli of colony formation. However, when an early factor such as IL-3 or GM-CSF is combined with a late factor, levels of colony formation equivalent to that seen with conditioned media having full activity is observed. Thus, differentiation appears to be dependent upon the dual activities of early and late factors.

Despite a clear requirement for both IL-3 or GM-CSF and Epo for the formation of erythroid colony forming units, published results indicate that IL-3 can down-modulate high affinity Epo receptors (4). Because the amount of IL-3 required to demonstrate down-modulation of the Epo receptor was higher than that reported by others who demonstrated functional full IL-3 activity in the presence of Epo, it is unclear whether this phenomenon is relevant in vivo.

Previous experiments in animals (22-26) suggest that under conditions of hematopoietic regeneration, optimal expansion of late progenitors could only occur in the presence of an adequate early progenitor pool. This then makes manipulations that result in the expansion of early hematopoietic progenitor pools extremely desirable. IL-3 has been shown to exert a differentiative and proliferative effect on early progenitor cells and at IL-3 concentrations which had little or no effect alone, Epo acted synergistically to induce proliferation and differentiation of erythroid progenitors. (27) By targeting a molecule with both early (IL-3) and late (Epo and G-CSF) activities to early progenitor cells, optimal expansion of a desired lineage should be possible.

SUMMARY OF THE INVENTION

The present invention concerns hybrid molecules comprising early and late differentiation factors produced by genetic manipulation. By covalently linking such factors the local concentration of the late factor is very high at the surface of a cell to which the early factor is bound. Additionally, if down-modulation is relevant in vivo, binding of late factors to any remaining low-affinity receptors, e.g. Epo receptors, could be enhanced, thus reducing the amount of late factor required to stimulate the cell. Furthermore, by linking an early factor with a late factor, such early factor may act more specifically to stimulate only the desired lineage, thus reducing any undesirable effects mediated by the early factor. Finally, it is considerably easier to produce and administer to a patient a single factor with two activities rather it would to produce and administer two separate factors.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a Western blot analysis of IL-3/Epo hybrid growth factors in CHO CM. CHO CM was collected from clones 23-10 (IL-3:Epo Flex), 5-4 (IL-3:Epo Short) and 17-3-1 (Epo:IL-3 Short). Hybrid growth factor concentrations were determined by ELISA assay. CM containing 74 ng of IL-3:Epo Flex (having a 23 aa flexible linker (lane 2), 73.5 ng of IL-3:Epo Short (having a short 2 aa linker) (lane 3), 80 ng of Epo:IL-3 Short (having a 3 aa linker) (lane 4) were subjected to SDS-PAGE (10-20% gel) electrophoresis and were assayed for Epo by Western blotting with a mouse anti-Epo polyclonal antisera as described in Example 7. Medium conditioned by CHO cells transfected with the vector pEe6 (lane 5) and rHu Epo 10 ng (lane 6), 20 ng (lane 7), 30 ng (lane 8), 70 ng (lane 9), and 100 ng (lane 10) were included. Molecular size markers in kilodaltons (lane 1).

Figure 2 shows AML193 cells proliferate in response to the IL-3 moiety of the hybrid growth factors. AML193 cells were grown to stationary phase and suspended in RPMI-1640 plus 10% FCS and growth

factor deprived for 16 hours. The indicated concentrations of growth factors were added for 42 hours followed by a 6 hour pulse of (^{3}H) thymidine as described in Example 7. NO GF (no growth factor); CHO CM (medium conditioned by CHO cells transfected with the vector pEe6); Epo (rHu Epo); IL-3 (rHu IL-3); IL-3:Epo Flex (CHO CM containing IL-3:Epo fusion protein with a 23aa flexible linker); IL-3:Epo Short (CHO CM containing IL-3:Epo fusion protein with a 2aa linker); Epo:IL-3 Short (CHO CM containing Epo:IL-3 fusion protein with a 3aa linker).

Figure 3 shows dose response of IL-3 adapted AML193 cells to the IL-3 moiety of the hybrid growth factors. IL-3 adapted AML 193 cells were grown to stationary phase and suspended in RPMI-1640 plus 10% FCS minus growth factor for 16 hours. Increasing concentrations of IL-3 and fusion proteins were added and the assay was carried out as described in Figure 2 and in Example 7. IL-3:Epo Flex (CHO CM containing IL-3:Epo fusion protein with a 23aa flexible linker); IL-3:Epo Short (CHO CM containing IL-3:Epo fusion protein with a 2aa linker); Epo:IL-3 Short (CHO CM containing Epo:IL-3 fusion protein with a 3aa linker).

Figure 4 shows FDC-P1/ER cells proliferate in response to the Epo moiety of the hybrid growth factors. FDC-P1/ER cells were grown to stationary phase and suspended in RPMI-1640 plus 10% FCS without growth factor for 16 hours. The indicated concentrations of growth factors were added for 42 hours followed by a 6 hour pulse of (^{3}H) thymidine as described in Example 7. Columns are labeled as described in Figure 2. WEHI3 CM (medium conditioned by murine WEHI3 cells which produce and secrete IL-3).

Figure 5 shows dose response of FDC-P1/ER cells to the Epo moiety of the hybrid growth factors. FDC-P1/ER cells were grown to stationary phase and suspended in RPMI-1640 plus 10% FCS and deprived of growth factor for 16 hours. Increasing concentrations of Epo and fusion proteins were added and the assay was carried out as described in Example 7. Hybrid growth factors are as designated in Figure 3.

Figure 6 shows IL-3 plus Epo responsiveness of IL-3 adapted TF-1 cells. TF-1 cells adapted for growth in IL-3 were grown to stationary phase and suspended in RPMI-1640 plus 10% FCS minus growth factor for 16 hours. Increasing concentrations of growth factors, 0.75 fmol/ml hybrid growth factors and 0.75 fmol/ml Epo plus 1.5 fmol/ml IL-3 (1), 1.5 fmol/ml hybrid growth factors and 1.5 fmol/ml of Epo plus 3.0 fmol/ml IL-3 (2), 3.0 fmol/ml hybrid growth factors and 3.0 fmol/ml Epo plus 6.0 fmol/ml IL-3 (3), were added and the assay was carried out as described in Example 7. Hybrid growth factors are as designated in Figure 3.

Figure 7 shows dose responsiveness of IL-3 adapted TF-1 cells to the hybrid growth factors. TF-1 cells adapted for growth in IL-3 were grown to log phase and suspended in RPMI-1640 plus 10% FCS minus growth factor for 16 hours. Increasing concentrations of hybrid growth factors were added and the cells were incubated for 8 hours. (^{3}H) Thymidine (1 μCi/well) was added and the incubation was continued for 16 hours. (A) Dose response to hybrid growth factor, concentrations of 0 to 30 fmol/ml. (B) Represents the same data as in A for concentrations of 0 to 1.875 fmol/ml to emphasize the differences between hybrid factors. Hybrid growth factors are as designated in Figure 3.

Figure 8 shows dose responsiveness of GM-CSF adapted TF-1 cells to the hybrid growth factors. TF-1 cells maintained in GM-CSF were grown to log phase and suspended in RPMI-1640 plus 10% FCS minus growth factor for 16 hours. Increasing concentrations of hybrid growth factors were added and the assay was carried out as described above for Figure 5. (A) Dose response to hybrid growth factor concentrations, of 0 to 30 fmol/ml. (B) Represents the same data as in A for concentrations of 0 to 1.875 fmol/ml to emphasize the differences between hybrid factors. Hybrid growth factors are as designated in Figure 3.

Figure 9 shows TF-1 cells proliferate in response to the IL-3 moiety of the IL-3/G-CSF hybrid growth factor. TF-1 cells were grown to stationary phase and suspended in RPMI-1640 plus 10% FCS deprived of

growth factor for 16 hours. The indicated concentrations of growth factors were added and the assay was carried out as described in Example 7. Factors are as designated in Figure 2 except, G-CSF (rHu G-CSF); IL-3/G-CSF (CHO CM containing IL-3/G-CSF fusion protein with a 10aa linker).

Figure 10 shows NFS-60 cells proliferate in response to the G-CSF moiety of the IL-3/G-CSF hybrid growth factor. NFS-60 cells were grown to stationary phase and suspended in RPMI-1640 plus 10% FCS minus growth factor for 16 hours. The indicated concentrations of growth factors were added and the assay was carried out as described in Example 7. Growth factors are as designated in Figures 2 and 9.

Figure 11 shows dose responsiveness of AML193 cells to the IL-3:G-CSF hybrid growth factor. AML193 cells were grown to stationary phase and suspended in RPMI-1640 plus 10% FCS deprived of growth factor for 16 hours. The indicated concentrations of growth factors were added and the assay was carried out as described in Example 7. Growth factors are as designated in Figures 2 and 9.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a recombinant hematopoietic molecule comprising at least a portion of a first hematopoietic molecule having early myeloid differentiation activity and at least a portion of a second hematopoietic molecule having late myeloid differentiation activity. This recombinant molecule has early myeloid differentiation activity associated with the first hematopoietic molecule and late myeloid differentiation activity associated with the second hematopoietic molecule. Within this application, "hematopoietic molecule" means a molecule which promotes and/or regulates hematopoiesis. Hematopoietic molecules exert such promotional or regulatory activities at different stages during hematopoiesis, such stages being referred to herein as early myeloid differentiation and late myeloid differentiation. Also within this application, "early myeloid differentiation activity" means the ability to promote the

differentiation, self-renewal, or proliferation of pluripotent myeloid cells, i.e., stem cells or colony forming unit, granulocyte-erythrocyte-monocyte-megacaryocyte, cells. Moreover, within this application, "late myeloid differentiation activity" means the ability to promote the maturation or differentiation of a lineage restricted myeloid cell, i.e., a myeloid precursor cell committed to a specific cell lineage such as erythrocytes, megakaryocytes, monocytes, neutrophils, eosinophils, and basophils.

In one embodiment of the invention, the first hematopoietic molecule is selected from the group consisting of IL-3 and GM-CSF. In another embodiment of the invention, the second hemopoietic molecule is selected from the group consisting of Epo, G-CSF, IL-5 and M-CSF. In a preferred embodiment of the invention, the portion of the first hematopoietic molecule is linked to the portion of the second hematopoietic molecule by an amino acid linker sequence comprising at least two amino acid residues.

Within the context of the present invention, it is understood that variations in proteins and nucleic acids exist among individuals, e.g. amino acid or nucleotide substitutions, deletions, insertions, and degree or location of glycosylation, and that functional derivatives resulting therefrom are included within the scope of the present invention.

In a preferred embodiment of the invention, the recombinant molecule comprises the entire amino acid sequence of human IL-3 (SEQ ID NO: 1). Moreover, the recombinant hematopoietic molecule may preferably comprise a 79 amino acid sequence derived from human IL-3 (SEQ ID NO: 2),i.e. residues 1-79 of SEQ ID NO: 1.

Further still, in yet another preferred embodiment of the invention, the recombinant molecule comprises the entire amino acid sequence of human erythropoietin (SEQ ID NO: 3). In still a further embodiment of the invention, the hemopoietic molecule comprises a 155 amino acid

sequence derived from human erythropoietin (SEQ ID NO: 4), i.e., residues 7-161 of SEQ ID NO: 3.

In another preferred embodiment of the invention, the recombinant hematopoietic molecule comprises the entire amino acid sequence of human G-CSF (SEQ ID NO: 5).

In one embodiment of the invention, the first hematopoietic molecule is IL-3 and the second hematopoietic molecule is erythropoietin. The first hematopoietic molecule, i.e. IL-3, may comprise the amino portion and the second hematopoietic molecule, i.e. Epo, may comprise the carboxyl portion of the recombinant molecule. Preferably, the recombinant hematopoietic molecule comprises the amino acid sequence from amino acid 1 to amino acid 302 of SEQ ID NO: 6. Also preferably, the recombinant hematopoietic molecule comprises the amino acid sequence from amino acid 1 to amino acid 321 of SEQ ID NO: 7. However, in another embodiment of the invention, the first hematopoietic molecule, i.e. IL-3, may comprise the carboxyl portion and the second hemopoietic molecule, i.e. Epo, may comprise the amino portion of the recombinant molecule. In a preferred embodiment of the invention, the recombinant molecule comprises the amino acid sequence from amino acid 1 to amino acid 303 of SEQ ID NO: 8. In yet another preferred embodiment, the recombinant molecule comprises the amino acid sequence from amino acid 1 to amino acid 322 of SEQ ID NO: 9.

In still a further embodiment of the invention, the first hematopoietic molecule is IL-3 and the second hematopoietic molecule is G-CSF. In one such embodiment, the first hematopoietic molecule comprises the amino portion and the second hematopoietic molecule comprises the carboxyl portion of the recombinant molecule. In yet a more specific embodiment, the recombinant molecule comprises the amino acid sequence from amino acid 1 to amino acid 317 of SEQ ID NO: 10.

The subject invention also provides nucleic acid molecules which encode the recombinant hematopoietic molecules of the subject invention. Examples of such nucleic acid molecules are SEQ ID NO: 11,

SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14 and SEQ ID NO: 15. Moreover, vectors which comprise the nucleic acid molecules of the subject invention are also disclosed. In one embodiment of the invention, the vector comprises a plasmid. Moreover, host vector systems for the production of a recombinant hematopoietic molecule of the present invention are provided which comprise a vector of the present invention in a suitable host, preferably a mammalian cell such as a CHO or COS cell. This host vector system may be grown under suitable conditions which permit the expression of the recombinant hematopoietic molecule, which may be recovered by purification techniques known in the art, e.g. ion exchange chromatography, affinity chromatography, and size exclusion chromatography.

The present invention further provides pharmaceutical compositions useful for treating patients suffering from anemias of various origins, e.g. renal failure, and AIDS. Moreover, these pharmaceutical compositions are useful for administering to patients for preoperative autologous blood donations, patients receiving or donating bone marrow for transplantation purposes, and patients undergoing cancer chemotherapy. These pharmaceutical compositions comprise effective hematopoiesis-promoting amounts of a recombinant molecule of the present invention and a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are known in the art and are disclosed in The Pharmacopeia of the United States and the National Formulary. Depending on the specific application contemplated, the pharmaceutical composition may be formulated as a solution, suspension, parenteral preparation, or spray. Parenteral preparations may include a vehicle such as specially distilled, pyrogen-free water, phosphate buffer, or normal saline. Oral and/or transmucosal dosage forms may comprise phospholipids, often in the form of liposomes.

Also provided is a method for treating a patient to promote hematopoiesis which comprises administering to the patient an effective hematopoiesis-promoting amount of a pharmaceutical composition of the present invention.

The recombinant hematopoietic molecules, nucleic acid molecules, pharmaceutical compositions and methods of the present invention will be better understood by reference to the following experiments and examples, which are provided for purposes of illustration and are not to be construed as in any way limiting the scope of the invention, which is defined by the claims appended hereto.

Examples

Construction of the hybrid protein genes: Genes encoding IL-3 (SEQ ID NO: 16), Epo (SEQ ID NO: 17) and G-CSF (SEQ ID NO: 18) were purchased from British Biotech. Ltd. These genes were utilized to construct three different hybrid hematopoietic proteins, i.e., IL-3:Epo, Epo:IL-3 and IL-3:G-CSF. In these hybrids the first named gene forms the amino portion and the second named gene the carboxyl portion of the hybrid protein.

Example 1

A nucleic acid molecule encoding an IL-3:Epo hybrid growth factor was constructed as follows: CSF, the native leader sequence of IL-3 was synthesized as 4 oligonucleotides (SEQ ID NOS: 19-22; see Table I) which represents both strands of the leader sequence. In addition, the 5′ end of the leader (SEQ ID NO: 19) encoded a convenient restriction enzyme overhang (EcoR1), although the EcoR1 site was not regenerated, in front of the ATG start codon. The 3′ end of the leader (SEQ ID NO: 21) included the first several amino acid codons of IL-3 and an SpeI overhang so that the annealed leader sequence could be easily ligated to IL-3, which was altered by British Biotech to include an SpeI site. The leader sequence was annealed and ligated to pKS (Stratagene Cloning Systems, Inc., San Diego, CA) cleaved with EcoR1 and SpeI. The resulting plasmid was designated pKSO. The IL-3 containing pUC18 plasmid obtained from British Biotech was cleaved with SpeI and NheI, then ligated to a linker oligonucleotide (complimentary oligonucleotide SEQ ID NOS: 23 and 24; see Table I) which contained the following three restriction sites: NheI, XbaI and

NcoI. Cleavage was then performed with SpeI and XbaI. The resulting 379 base pair fragment was then ligated to PKSO cleaved with

SpeI and XbaI. The resulting plasmid (pKSOIL-a) contained the IL-3 leader, the IL-3 gene and a small linker fragment.

The Epo gene was inserted into pEe6 (Celltech, Ltd., Slough, U.K.), a mammalian expression vector which contains the human Cytomegalovirus promoter, a polylinker region and a poly-A addition site in addition to ampicillin resistance and a bacterial origin of replication, by cleaving the Epo containing plasmid obtained from British Biotech with HindIII and BamHl. Epo was then cleaved with NcoI. The same linker comprising oligonucleotide SEQ ID NOS: 23 and 24 as described earlier was ligated to Epo and then cleaved with XbaI to yield the entire Epo gene. This was then ligated to XbaI and BclI cleaved pEe6 to yield pEe6 containing the Epo gene (pEepo). PKSOIL-a was cleaved with EcoRV and an XbaI linker was ligated to the blunt ends followed by cleavage with XbaI, which released the IL-3 gene with the leader sequence. This was then ligated to XbaI cleaved Peepo to yield a plasmid containing an entire hybrid protein gene (pEepie-a) (see SEQ ID NO: 11 for the structure of the inserted hybrid gene, designated herein IL-3:Epo Short). The glutamine synthetase (gs) gene was then inserted into the BamHl site of pEepie-a to yield pEepogs-a or pEpogs-b, depending upon the orientation of the gs gene. Glutamine synthetase confers resistance to methionine sulphoximine (MSX) in order to select cells which have taken up the plasmid after transfection. After the plasmid was constructed a large batch was grown, purified by CsCl ultracentrifugation, and used for transfection. At each step in this process all ligation joints between fragments were analyzed by DNA sequence analysis in order to assure that there were no changes that would cause frameshifts and prevent the hybrid gene from being expressed.

To construct a nucleic acid molecule encoding an IL-3:Epo hybrid growth factor with a longer linker sequence separating IL-3 and Epo, pEepie-a was cleaved with NheI and annealed oligonucleotide SEQ ID

NOS: 25 and 26 (see Table I) were ligated into the cleaved plasmid. This linker encodes the flexible amino acid sequence Gly Ser Gly Ser Gly Ser (SEQ ID NO: 27). Clones with the insert in the proper orientation were selected by probing colonies with the junction oligonucleotide SEQ ID NO: 28 (see SEQ ID NO: 14 for the structure of the inserted hybrid gene, designated herein IL-3:Epo Flex). The glutamine synthetase gene was then added to the construct as described above.

Example 2

A nucleic acid molecule encoding an IL-3:G-CSF hybrid growth factor was constructed as follows: pUC18 containing G-CSF (British Biotech) was cleaved with HindIII. A linker composed of an overhanging XbaI site, a NotI site and an overhanging HindIII site (oligonucleotide SEQ ID NOS: 29 and 30; see Table I) was ligated to the pUC18:G-CSF. This was then cleaved with XbaI and BamHl which released the entire G-CSF gene. The G-CSF fragment was then inserted into XbaI and BclI cleaved pEe6 (pEe6:G-CSF). IL-3 with its signal sequence was removed from the IL-3:Epo plasmid pEepogs-a as an XbaI fragment. This IL-3 fragment was then inserted into XbaI cleaved pEe6-G-CSF. After restriction analysis, a plasmid containing the IL-3 gene in the proper orientation was obtained (pEG11), this plasmid encoded a gene capable of expressing IL-3 and G-CSF as a hybrid protein (see SEQ ID NO: 13 for the structure of the inserted hybrid gene, designated herein IL-3:G-CSF). The gs gene was inserted into this plasmid as described in Example 1 above to yield plasmids pEG13 and pEG14, depending upon the orientation of the gs gene.

Example 3

A nucleic acid molecule encoding an Epo:IL-3 hybrid growth factor was constructed by first synthesizing the native Epo signal sequence as oligonucleotide SEQ ID NOS: 31-36 (see Table I). These were annealed to yield an overhanging 5′ XhoI sequence and a 3′ PstI sequence. These were then ligated and subcloned as an XhoI/PstI fragment

(pEpol). In order to obtain the proper reading frame and signal sequence processing site, the plasmid containing the signal sequence was cleaved with PstI and the 3' overhang left by PstI was enzymatically removed with T4 polymerase. This was then cleaved with BamHl. The Epo gene was then amplified by PCR as a fragment with a 5' blunt end using oligonucleotide SEQ ID NO: 37 as a primer and a 3' BamHl end using oligonucleotide SEQ ID NO: 38 as a primer. This fragment was then ligated into pEpol to yield a complete Epo gene with its leader sequence. PCR was used to amplify the Epo gene with its signal sequence as an (5') XbaI and (3') NotI fragment using oligonucleotide SEQ ID NOS: 39 and 40 as primers. This was then digested with XbaI and NotI. At the same time, a purified IL-3 fragment was amplified by PCR as a (5') NotI and (3') BamHl fragment using oligonucleotide SEQ ID NOS: 41 and 42, followed by digestion with NotI and BamHl. These two fragments were ligated to pEe6 cleaved with Xbal and BclI to yield a full length hybrid gene encoding both Epo and IL-3 (pEG16) (see SEQ ID NO: 12 for the structure of the inserted hybrid gene, designated herein Epo:IL-3 Short). The gs gene was inserted as described in Example 1 above to yield pEG17 and pEG18, depending upon the orientation of the gs gene.

A flexible linker is inserted into Epo:IL-3 by cleaving pEG17 or pEG18 with NotI. Annealed oligonucleotide SEQ ID NOS: 43 and 44 are then ligated into the cleaved plasmid. Clones with the insert in the proper orientation are selected by probing colonies with a junction oligonucleotide as described above (see SEQ ID NO: 15 for the structure of the inserted hybrid gene.)

TABLE I

OLIGONUCLEOTIDES

All oligonucleotides are listed in the 5' to 3' orientation:

AATTGCCGCC ACCATGAGCC GCCTGCCCGT CCTGCTCCT (SEQ ID NO: 19)

GCTCCAACTC CTGGTCCGCC CCGGACTCCA AGCTCCCATG ACCCAGACAA (SEQ ID NO: 20)

CTAGTTGTCT GGGTCATGGG AGCTTGGAGT CCGGGGCGG (SEQ ID NO: 21)

ACCAGGAGTT GGAGCAGGAG CAGGACGGGC AGGCGGCTCAT GGTGGCGGC (SEQ ID NO: 22)

CTAGCGATCT TTCTAGA (SEQ ID NO: 23)

CATGTCTAGA AAGATCG (SEQ ID NO: 24)

CTAGAAGCGG CCGCA (SEQ ID NO: 29)

TTCGCCGGCG TTCGA (SEQ ID NO: 30)

TCGAGCCATG GGGGTGCACG AATGTCCT (SEQ ID NO: 31)

GCCTGGCTGT GGCTTCTCCT GTCCCTGCTG TC (SEQ ID NO: 32)

GCTCCCTCTG GGCCTCCCAG TCCTGGGCTG CA (SEQ ID NO: 33)

GCCCAGGACT GGGAGGCCCA GAGGGA (SEQ ID NO: 34)

GCGACAGCAG GGACAGGAGA AGCCACAGCC AGGCAGGACA TT (SEQ ID NO: 35)

CGTGCACCCC CATGGC (SEQ ID NO: 36)

GCCCCACCAC GCCTCATCTG T (SEQ ID NO: 37)

GAATTCGGAT CCTTATCATC T (SEQ ID NO: 38)

CTAGTCTCTA GAATGGGGGT CCACGAATGT (SEQ ID NO: 39)

AGCCATGGCG GCCGCTCTGT CCCCTGTCCT (SEQ ID NO: 40)

GACAGAGCGG CCGCCATGGC TCCCATGACC (SEQ ID NO: 41)

GAATTCGGAT CCTTACTAAA AGATCGCTAG (SEQ ID NO: 42)

CTAGCGTCCG GAGGCGGTGG CTCGGGCGGT GGCGGCTCGG GTGGCGGCG GCTCTGCG (SEQ ID NO: 25)

CTAGCGCAGA GCCGCCGCCA CCGCAGCCGC CACCGCCCGA GCCACCGCC TCCGGACG (SEQ ID NO: 26)

TTGTCGCTAG CGTCCGGAGG C (SEQ ID NO: 28)

GGCCGCTTCC GGAGGCGGTG GCTCGGGCGG TGGCGGCTCG GGTGGCGGC GGCTCTGC (SEQ ID NO: 43)

GGCCGCAGAG CCGCCGCCAC CCGAGCCGCC ACCGCCCGAG CCACCGCCT CCGGCAGC (SEQ ID NO: 44)

Example 4

Transfection of the hybrid gene containing plasmids. All transfections were performed using the Lipofectinç transfection kit (Bethesda Research Labs, Gaithersburg, MD) using 15-30 mg. of purified plasmid DNA (pEepogs-a, pEepogs-b, pEG13, pEG14, pEG17, and pEG18). The following alterations were made to the protocol provided by the company: the growth medium in these experiments was GMEM-S and the CHO-K1 cells were incubated in the presence of 10% CO_2; after addition of the lipofectin:DNA complex, cells were incubated without selection for 24 hours. The cells were transferred to GMEM-S supplemented with 25 mM MSX after 24 hours. The MSX concentration was subsequently increased to 50 mM after one week. Cloning rings were used to subclone MSX resistant colonies and each of these colonies was placed into an individual well of a 24 well plate. Selected clones were incubated in the absence of MSX to insure that the hybrid protein gene was stably integrated. Strongly positive clones were grown in large cultures to provide larger amounts of hybrid proteins for further analysis.

Example 5

Assays for hybrid protein production. Cell supernatants from transfected or control cells were assayed using several different assays. In order to demonstrate Epo production, an RIA kit for Epo was used (Incstar Corp., Stillwater, MN). The presence of IL-3 was determined using an ELISA assay in which the capture antibody was a polyclonal goat anti-IL-3 (R&D Systems, Minneapolis, MN) and the probe antibody was a murine anti-IL-3 monoclonal. Goat anti-mouse conjugated to horseradish peroxidase followed by suitable substrate was used to detect the presence of the monoclonal anti-IL-3. A very similar assay was used to demonstrate the presence of the hybrid proteins except that a murine anti-Epo monoclonal or anti-G-CSF monoclonal was used in place of anti-IL-3 monoclonal. Additionally, IL-3:Epo Short was analyzed by Western blot analysis. The blot was probed with antibody to Epo and then with ^{125}I goat anti-mouse. A

single broad band appeared on the autoradiogram with a molecular weight of slightly more than 50,000 daltons.

Example 6

Cellular assays. Epo and/or IL-3 dependent and responsive cell lines were used to test the biological activities of the hybrid proteins. B6SUtA (5) is a multipotential hematopoietic progenitor cell line established from nonadherent cell populations removed from continuous B6.S mouse bone marrow culture. This cell line demonstrates absolute dependence upon a source of growth factor(s). In response to Epo a population of the cells synthesize hemoglobin. Studies of globin expression indicated that the globin programs of B6SUtA cells are similar to those of erythroid progenitors at the period of transition from the yolk sac to fetal liver erythropoiesis. TF-1 (6) it is a cell line of immature erythroid origin established from a patient with erythroleukemia. The cell line shows complete dependency on GM-CSF or IL-3. Epo sustains short-term growth of TF-1 and will induce hemoglobin synthesis in a very small population of cells (8%). Hemin and w-aminolevulinic acid induce hemoglobin synthesis in most of the cells.

Human IL-3 will not bind the murine IL-3 receptor, therefore experiments that were done with B6SUtA cells measured only the functionality of the Epo moiety of the hybrid. B6SUtA cells are carried in murine IL-3. In each experiment, they are washed thoroughly and set up with growth factors at 10^5 cells/ml. Cell growth and hemoglobin content were monitored on days 3 and 6 of each experiment. Cells grown in the presence of concentrated (10X) CHO conditioned medium (CM) containing IL-3:Epo Short at a final concentration equivalent to 4.8 units/ml of Epo grew as well as cells grown in an equivalent amount of recombinant human (rHu) Epo. The percentage of cells which synthesized hemoglobin in response to the CHO-IL-3:Epo Short CM was always four times that of cells exposed to rHu Epo. B6SUtA cells grown in the presence of rHu IL-3 and rHu Epo grew as well as cells grown in the presence of IL-3:Epo Short and

induced hemoglobin synthesis in the same percentage of cells as did rHu Epo. Cells exposed to recombinant murine IL-3 (rMu IL-3) and rHu Epo grew similarly to cells exposed to rMu IL-3 alone and neither effectively induced cells to synthesize hemoglobin. Concentrated control CHO CM did not support the growth of B6SUtA cells nor did it induce hemoglobin synthesis. CHO CM plus rHu Epo supported cell growth and hemoglobinization as well as CHO-IL-3:Epo Short CM.

CHO-IL-3:Epo Short CM as well as CHO-rHu IL-3 CM both supported growth of human TF-1 cells. Control CHO CM supported only limited growth of the TF-1 cells.

Discussion

The above-mentioned results demonstrate that a hybrid protein comprising two growth factors can be expressed in mammalian cell culture systems. In vitro assays of IL-3:Epo Short indicate that this hybrid protein has the activities of both IL-3 and Epo. The therapeutic application of such hybrid factors has advantages over using two factors separately simply in terms of patient administration, and moreover since the production, purification and formulation of one factor is less labor intensive than for two separate factors.

Example 7

Factor Dependent Cell Lines and Culture Media - The GM-CSF/IL-3/Epo dependent human TF-1 cell line and the G-CSF dependent murine NFS-60 cell line were grown and maintained as described (7,8,). The GM-CSF dependent human cell line AML 193 (9) was adapted for growth in IL-3 by continuous culture of the cells in RPMI-1640 plus 10% FCS supplemented with rHu IL-3 for 6 weeks. The TF-1 derived cell line, TF-136 was selected by continuous culture of the TF-1 line in RPMI-1640 plus 10% FCS supplemented with 5ng/ml of rHu IL-3 for 6 months, followed by single cell suspension cloning of the resultant IL-3 dependent cells. The Epo dependent murine cell line, FDC-P1/ER,

was derived from the IL-3 dependent line, FDC-P1, by introduction of the murine Epo receptor into these cells. (10) FDC-P1/ER cells are maintained in RPMI-1640 plus 10% FCS supplemented with 1 unit/ml of rHu Epo. Recombinant human Epo was obtained from Ortho Biologicals, Inc (Raritan, NJ). Recombinant human IL-3, rHu G-CSF and rHu GM-CSF were purchased from R & D Systems (Minneapolis, MN).

Capture ELISA Assay - ELISA plate was coated with 5 μg/200 μl/well of goat anti-human IL-3 (R & D Systems) in PBS at 40°C overnight. Excess antibody was removed by washing with PBS. Blocking was carried out with 300 μl/well of 1% non-fat milk in PBS for 1 hour at 37°C followed by washing with 0.05% Tween™ in PBS. Samples were then incubated with the IL-3 antibody for 1 hour at 37°C in 0.5% non-fat milk, 0.025% Tween™. Following extensive washing, the second antibody, a mouse anti-Epo monoclonal (Genzyme, Cambridge, MA), was added to the plate which was incubated for 1 hour at 37°C. The plate was washed and incubated with conjugate antibody (Goat anti-mouse-horseradish peroxidase) for 30 minutes at 37°C. Color development was carried out with the addition of o-phenylenediamine/H_2O_2 at room temperature (RT) for 30 minutes. The reaction was stopped with 1N H_2SO_4 and the samples were read at 495 nm.

Gene Amplification- CHO cell lines producing significant amounts of the hybrid growth factors were isolated and 10^6 cells were plated in a 75 mm T-flask in GMEM-S medium containing various concentrations of methionine sulphoximine (MSX), ranging between 100μM and 500μM. Colonies resistant to the highest MSX concentration (IL-3:Epo Flex 200μM; IL-3:Epo Short 250μM; Epo:IL-3 Short 250μM; IL-3:G-CSF 250μM) were isolated and expanded. Those clones producing the highest levels of hybrid growth factors as determined by Epo or G-CSF ELISA assay (Amgen) were used for subsequent studies. IL-3:Epo Flex (clone 23-10); IL-3:Epo Short (clone 5-4); Epo:IL-3 Short (17-3-1).

Cell Proliferation Assays - Factor dependent cells were grown to stationary phase, washed, and incubated for 16 hours in media plus 10% FCS deprived of growth factor. The cells were plated at a

concentration of 2 X 10^5 cells/ml in a 96 well microtiter plate (200 μl/well) with and without growth factor. Recombinant human growth factors were diluted into CHO conditioned medium (CM) before addition to cells. Following incubation for 42 hours, (^{3}H) thymidine (1 μCi/well; New England Nuclear, Boston, MA) was added and the cells were incubated for another 6 hours. The cells were then hypotonically lysed and harvested onto glass fiber filters. The filters were washed with distilled water, dried and counted in liquid scintillation fluid.

Bone Marrow Cultures - Informed consent was obtained prior to aspirating bone marrow from normal volunteers. Aspirated bone marrow was diluted 1:1 in α- medium without nucleosides containing preservative-free sodium heparin. A single cell suspension was prepared, layered, over an equal volume of Ficoll-Hypaque (sp gr 1.077 g/ml) and then centrifuged for 25 minutes at 1,500 rpm at 40°C. The light-density mononuclear cells were collected and washed and diluted to 5 X10^5 cells/ml with Iscove's modified Dulbecco's medium plus 20% modified FCS (Gibco BRL). Cells (1.25 X 10^5 /ml) were plated in 0.8% methylcellulose supplemented with various concentrations of rHu Epo, rHu IL-3 and hybrid growth factors. Cultures were incubated for either 7 or 14 days in a humidified atmosphere with 5% CO_2 at 37°C. Colonies were counted at day 7 for CFU-E and at day 14 for BFU-E under an inverted microscope.

Western Blot Analysis - CHO CM containing approximately 75 ng of IL-3:Epo fusion protein was electrophoresed on a 10-20% gradient SDS PAGE gel (Integrated Separations Systems) under reducing and denaturing conditions. Samples were loaded in 0.0625 M Tris-HCl (pH 6.8), 2% SDS, 5% 2-mercaptoethanol, 10% glycerol and 0.002% bromophenolblue following heat treatment at 100°C for 3 minutes. The proteins were transferred to nitrocellulose (Bio Rad) in 25 mM Tris, 129 mM glycine, pH 8.3, 20% methanol, at 150 V, constant power, for 90 minutes. The transfer efficiency was monitored by visual examination of the completeness of transfer of prestained molecular weight markers (Bio Rad). The nitrocellulose membrane was incubated in PBS containing 3% BSA for 1 hour at room temperature and subsequently washed in PBS

containing 0.5% Tween (PBS-T) for 5 minutes at room temperature. The membrane was probed with primary anti-Epo anti-sera in 3% BSA in PBS. Excess antibody was removed by 3, 5 minute room temperature washes in PBS-T. The nitrocellulose membrane was then probed with a secondary antibody conjugate (Goat anti-Rabbit IgG/ Alkaline Phosphatase, Bio Rad) for 1 hour at room temperature. Excess secondary antibody was removed by two washes with PBS-T as above. Color development was carried out by incubation with color reagents (Bio Rad) in alkaline phosphatase buffer (100 mM Tris HCl, pH 9.5, 100 mM NaCl, 5 mM $MgCl_2$). The reaction was stopped by immersion of the membrane in cold (4°C) distilled H_2O.

Results and Discussion

Hybrid growth factor plasmid amplification. Individual transfected CHO cell clones producing significant amounts of the desired hybrid growth factor were identified by ELISA capture assay, Table II. The clones were plated out and placed in medium with increasing concentrations of MSX, ranging between 100 μM and 500 μM. Colonies surviving at the highest concentration of MSX were isolated and grown to confluence. Serum and drug-free medium was then added to the cells and collected after 4 days. At the time of collection fresh serum and drug-free medium was added to the cells. A total of 3 collections were taken. The amount of hybrid growth factor produced in the collections was determined by Epo or G-CSF ELISA assay (Table III) and appropriate collections were pooled. The pooled CM was used as a source of hybrid growth factors in all cellular assays.

TABLE II

Hybrid Growth Factor	OD (495 nm)
CHO CM*	0.24
IL-3:Epo Flex	2.44
IL-3:Epo Short	2.46
Epo:IL-3 Short	1.59
IL-3:G-CSF	2.40

* Conditioned medium from CHO cells transfected with the vector pEe6.

TABLE III

Hybrid Growth Factor	Collection	Concentration (μg/ml)*
IL-3:Epo Flex	1st	3.0
	2nd	4.2
	3rd	3.3
IL-3:Epo Short	1st	1.5
	2nd	5.8
	3rd	6.7
Epo:IL-3 Short	1st	26.7
	2nd	53.3
	3rd	58.7
IL-3:G-CSF	1st	2.2
	2nd	2.0
	3rd	2.0

* Concentrations were determined by Epo and G-CSF ELISA Assay.

Detection of hybrid growth factor production. In order to confirm that the IL-3 and Epo detected in the ELISA capture assays were being produced in the form of a fusion protein, Western blot analysis was performed. Conditioned medium from CHO cells transfected with IL-3:Epo Flex cDNA (Figure 1, lane 2), IL-3:Epo Short cDNA (Figure 1, lane 3) and Epo:IL-3 Short cDNA (Figure 1, lane 4) were probed with mouse anti-Epo polyclonal anti-sera. Immunoreactive material corresponding to a molecular weight of approximately 50,000 daltons,

the expected size of the IL-3:Epo and Epo:IL-3 hybrid growth factors, was detected in each sample. Comparison with increasing concentrations of rHu Epo (Figure 1, lanes 6-10) indicated that the antibody used in this analysis recognizes the Epo moiety of the fusion proteins efficiently.

IL-3 bioactivity of the IL-3:Epo and Epo:IL-3 hybrid growth factors. To determine whether the IL-3 moiety of the IL-3:Epo and Epo:IL-3 hybrid growth factors was functional, its ability to support growth of the IL-3-dependent human cell line, AML193, was evaluated (Figure 2). As Epo does not support growth of these cells (Figure 2), only IL-3 activity was measured in this assay system. CHO CM containing rHu IL-3 and levels of hybrid growth factors sufficient to support maximal proliferation were added to the culture medium. The cells were then pulsed with (^{3}H) thymidine and the radioactivity incorporated into the DNA was used as a measure of cell growth. Cells exposed to CHO CM containing no growth factors, supported the proliferation of AML193 cells to the same extent as did cells grown in medium alone. Each of the fusion proteins when present in excess, supported the growth of AML193 cells in a manner equivalent to that of rHu IL-3.

The functional activity of the IL-3 portion of the IL-3:Epo and Epo:IL-3 hybrid growth factors was further evaluated by comparing the fusion proteins to rHu IL-3 in dose response experiments (Figure 3). The incorporation of (^{3}H) thymidine into AML193 DNA was again used as a measure of cell proliferation. When IL-3 was located at the N-terminus of the hybrid growth factor protein (IL-3:Epo), its ability to support AML193 proliferation was equivalent to that of rHu IL-3 (ED50 = 5 fmol/ml). Size (2 aa versus 23 aa) and flexibility of the linker did not greatly impact the function of the IL-3 moiety. However, when IL-3 was located at the C-terminus of the fusion protein (Epo:IL-3), its ability to support the growth of AML193 cells was less (ED50 = 200 fmol/ml) than that of rHu IL-3 and the IL-3:Epo hybrid factors. These results suggest that linkage of IL-3 at the N-terminus interferes with function while linkage at the C-terminus does not. It has previously been reported that modification of the C-terminus of

murine IL-3 did not interfere with its activity (11). Therefore, it should be possible to target any molecule or compound of interest to cells expressing the IL-3 receptor through linkage to IL-3 at its C-terminus.

Epo bioactivity of the IL-3:Epo and Epo:IL-3 hybrid growth factors. To determine whether the Epo moiety of the hybrid growth factors was functional, its ability to support the growth of the Epo-dependent murine cell line FDC-P1/ER, was evaluated (Figure 4). This line derived from FDC-P1 cells expresses the murine Epo receptor (10), and is dependent on either murine IL-3 or Epo (murine and human) for growth (Figure 4). As IL-3 is a species specific growth factor, murine IL-3-dependent cells do not respond to human IL-3 (12). Therefore, when using the FDC-P1/ER cell line to evaluate functionality, only the activity of the Epo moiety is measured. CHO CM containing rHu Epo and levels of hybrid growth factors sufficient to support maximal proliferation were added to the culture medium. The cells were then pulsed with (^{3}H) thymidine and the radioactivity incorporated into the DNA was used as a measure of cell growth. Cells exposed to CHO CM which did not contain cytokines did not support the proliferation of FDC-P1/ER cells. Each of the fusion proteins when present in excess, stimulated the growth of FDC-P1/ER cells to the same extent as did rHu Epo. (Figure 4)

The biological function of the Epo portion of the IL-3:Epo and Epo:IL-3 hybrid growth factors was further analyzed by comparing the fusion proteins to rHu Epo in dose response experiments (Figure 5). The incorporation of (^{3}H) thymidine into FDC-P1/ER cells was used as a measure of cell proliferation. Each of the hybrid growth factors was equivalent to rHu Epo in ability to stimulate proliferation of FDC-P1/ER cells (ED50 = 50 fmol/ml) . Size (2-3 aa versus 23 aa) and flexibility of the linker, as well as the orientation of Epo within the protein (N-terminus versus C-terminus) did not alter function. Evidence exists suggesting that the N-terminus of Epo is not involved in receptor binding as a monoclonal antibody directed toward the N-terminus of Epo does not neutralize its activity (13). The results

presented here suggest that linkage of Epo to a second protein does not impair its ability to bind its receptor or transduce a signal. Epo could therefore be useful as a carrier protein which would target a molecule or compound of interest to those cells expressing the Epo receptor.

IL-3 plus Epo bioactivity of the IL-3:Epo and Epo:IL-3 hybrid growth factors. In order to study the effects of IL-3 and Epo in combination, proliferation of a human cell line, TF-1 (7), dependent on IL-3 and Epo for growth was measured. This experiment was done on a cytokine weight basis and the results are represented on a molar basis (Figure 6). rHu IL-3 (R & D Systems) made in E. coli is nonglycosylated. rHu Epo and hybrid growth factors made in CHO cells are glycosylated. Therefore, when equal weights of the growth factors were added to the cell culture medium, approximately twice the number of unglycosylated molecules of IL-3 were added as compared to glycosylated Epo and hybrid growth factor molecules.

CHO CM containing rhu IL-3 plus rHu Epo and levels of hybrid growth factors which support suboptimal proliferation of TF-1 cells adapted for growth in IL-3 were added to the culture medium. Cell growth was monitored by radioactivity incorporated into the DNA. (Figure 6) The activities of IL-3 plus Epo were not synergistic in this cell line, nor were they additive. At these low levels, the activities of the IL-3:Epo Flex and IL-3:Epo Short fusion proteins were comparable to those of a mixture of the two cytokines. Epo:IL-3 Short activity was again reduced in comparison to that of the IL-3:Epo hybrid growth factors and the combination of IL-3 plus Epo. This is likely to be due to decreased IL-3 activity.

The biological activity of the IL-3:Epo and Epo:IL-3 hybrid growth factors was further evaluated in dose response experiments (Figures 7 & 8). TF-1 cells adapted for optimal growth in IL-3 were exposed to CHO CM containing hybrid growth factors (Figure 7). Each of the fusion proteins when present in excess were able to support growth of the cells to the same extent (Figure 7A). At lower doses, the IL-

3:Epo Flex protein appeared to be slightly more potent than the short linkered hybrids (IL-3:Epo Flex ED50 = 0.37 fmol/ml; IL-3:Epo Short ED50 = 0.75 fmol/ml; Epo:IL-3 Short ED50 = 0.9 fmol/ml) (Figure 7B). This result was more pronounced in experiments done with TF-1 cells adapted for optimal growth in GM-CSF (Figure 8). The IL-3:Epo Flex protein was dramatically more potent than the hybrid growth factors containing short linkers (IL-3:Epo Flex ED50 = 0.07 fmol/ml; IL-3:Epo Short and Epo:IL-3 Short ED50 = 0.75 fmol/ml (Figure 8B). When present in excess with the GM-CSF adapted TF-1 cells, each of the fusion proteins stimulated cell proliferation to a similar extent (Figure 8A). These results suggest that when IL-3 and Epo are fused, the 23 aa flexible linker allows more efficient receptor interaction than does a short (2-3 aa) linker.

It appears that induction of receptor expression is possible by growing a cell in the presence of a cytokine whose receptor it has the potential to express. An up regulation of Epo receptor expression has been reported in IL-3-dependent cells transferred into medium supplemented with Epo (14). Thus, it is likely that growing TF-1 cells in IL-3 or GM-CSF, preferentially increases the appearance of cell surface IL-3 or GM-CSF receptors. Several research groups (15-20) have observed a subset population of GM-CSF and IL-3 receptors on primary human cells and hematopoietic cell lines capable of binding both GM-CSF and IL-3. It has been suggested that a single accessory molecule preferentially interacts with this subset of GM-CSF/IL-3 receptors allowing the transduction of signal. Our results raise the possibility that GM-CSF could be inducing the expression of an accessory molecule in TF-1 cells which may be important for binding and could possibly link IL-3:Epo signal transduction. This protein could be identical to the GM-CSF/IL-3 receptor accessory protein.

Erythroid colony formation stimulated by IL-3:Epo and Epo:IL-3 hybrid growth factors. To assess the biological activity of the fusion proteins on normal hematopoietic progenitor cells, analysis of the formation of erythroid (BFU-E and CFU-E) colonies from nonadherent mononuclear human bone marrow cells was performed. (Table IV) As was

observed with the cell lines, the Epo moiety of each of the fusion proteins was equally active (CFU-E formation) on bone marrow progenitor cells. The IL-3:Epo Flex protein was the most active hybrid growth factor while the Epo:IL-3 Short protein was the least. (It stimulated two-thirds the number of BFU-E as did the IL-3:Epo Flex.) These results suggest that the IL-3:Epo Flex fusion protein may have significant clinical benefits where indications for combination therapy with IL-3 and Epo may prove efficacious.

TABLE IV

Growth Factors	BFU-E[a,b]	CFU-E[c]
No Factors	-	-
0.2 pmol/ml IL-3	-	-
0.1 pmol/ml Epo	+	+++
0.2 pmol/ml IL-3 & 0.1 pmol/ml Epo	+++	+++
0.1 pmol/ml IL-3:Epo Flex	+++	+++
0.1 pmol/ml IL-3:Epo Short	++	+++
0.1 pmol/ml Epo:IL-3 Short	+	+++

a Mononuclear human bone marrow cells were used as a target cell population.

b BFU-E were counted 14 days after plating.

c CFU-E were counted 7 days after plating.

IL-3 bioactivity of the IL-3:G-CSF hybrid growth factor. To determine whether the IL-3 moiety of the IL-3:G-CSF hybrid growth factor was functional, its ability to support growth of the IL-3-dependent human cell line TF-1, was evaluated in a dose response experiment (Figure 9). Quantitation of IL-3:G-CSF protein in CHO CM was performed using a G-CSF ELISA assay in which the standard is unglycosylated G-CSF. Since the IL-3:G-CSF fusion protein is glycosylated, measurements are approximate. G-CSF does not support growth of TF-1 cells (Figure 9), therefore, the only activity measured in this assay system was IL-3.

CHO CM containing rhu IL-3, rHu G-CSF, and IL-3:G-CSF hybrid growth factor were added to the culture medium. The radioactivity incorporated into the DNA was used as a measure of cell proliferation. CHO CM did not support growth of TF-1 cells. The mixture of rhu IL-3 plus rHu G-CSF stimulated proliferation to the same extent as did rhu IL-3 alone. The IL-3:G-CSF hybrid growth factor induced a dose response similar to that observed with IL-3.

G-CSF bioactivity of the IL-3:G-CSF hybrid growth factor. To evaluate the biological function of the G-CSF moiety of the IL-3:G-CSF hybrid growth factor, its ability to stimulate proliferation of the murine cell line, NSF-60, was tested. (Figure 10) G-CSF, unlike IL-3 is not species specific, therefore, human G-CSF will actively support growth of murine cells (21). Cells exposed to CHO CM containing no growth factors, supported the proliferation of NSF-60 cells to the same extent as did cells grown in medium alone. The IL-3:G-CSF hybrid growth factor stimulated growth in a dose dependent manner equivalent to that observed with G-CSF.

IL-3 plus G-CSF bioactivity of the IL-3:G-CSF hybrid growth factor. The biological function of the IL-3:G-CSF hybrid growth factor was evaluated by its ability to support growth of an IL-3-, G-CSF-dependent human cell line, AML193. CHO CM containing rHu IL-3, rHu G-CSF and IL-3:G-CSF hybrid growth factor were added to the culture medium. Cell proliferation was monitored by incorporation of radioactivity into the DNA. (Figure 11). Both IL-3 and G-CSF supported growth of this cell line in a dose dependent manner. The two cytokine activities were not synergistic, nor were they additive. The IL-3:G-CSF hybrid growth factor stimulated AML193 proliferation to a greater extent than did the mixture of the two cytokines.

REFERENCES

1. Sonoda, et al., Proc. Natl. Acad. Sci. USA, 85:4630-4364 (1988).

2. Migliaccio, et al., Blood, Vol. 72, No. 3, 844-851 (1988).

3. Sieff, et al., Blood, Vol.73, No. 3, 688-693 (1989).

4. Fraser, et al., Exp. Hematol., 16: 769-775 (1988).

5. Enver, et al, Proc.Natl. Acad. Sci. USA, 85:9091-9095 (1988).

6. Kitamura, et al., J. Physiol., 140:323-334 (1989).

7. Kitamura, et al., J. Cell. Physiol., 140 : 323-334 (1989).

8. Holmes, et al., Proc. Natl. Acad. Sci. USA, 82 : 6687-6691 (1985).

9. Lange, et al., Blood, 70 : 192-199 (1987).

10. Carroll, et al., J. Biol. Chem., in press (1991).

11. Dunbar, et al., Science, 245 : 1493-1496 (1989).

12. Yang, et al., Cell, 47 : 3-10 (1986).

13. Sue, et al., Proc. Natl. Acad. Sci. USA, 80 : 3651-3655 (1983).

14. Sakaguchi, et al., Biochem. Biophys. Res. Commun., 146 : 7-12 (1987).

15. Park, et al., J. Biol. Chem., 264 : 5420-5427 (1989).

16. Gesner, et al., J. Cell. Physiol., 136 : 493-499 (1988).

17. Elliot, et al., Blood, 74 : 2349-2359 (1989).

18. Lopez, et al., Proc. Natl. Acad. Sci. USA, 86 : 7022-7026 (1989).

19. Budel, et al., Blood, 75 : 1439-1445 (1990).

20. Cannistra, et al., J. Biol. Chem., 265 : 12656-12663 (1990).

21. Souza, et al., Science, 232 : 61-65 (1986).

22. Umemura et al., Proc. Natl. Acad. Sci. USA, 85 : 9278-9282 (1988).

23. Udupa, et al., Blood, 53 : 1164-1171 (1979).

24. Papayannopoulou, et al., J. Clin. Invest., 51 : 1179-1185 (1972).

25. Migliaccio, et al., Blood, 72 : 944-951 (1988).

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Rosen, Jonathan I.

(ii) TITLE OF INVENTION: HYBRID GROWTH FACTORS

(iii) NUMBER OF SEQUENCES: 44

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Robert L. Minier
(B) STREET: 1 Johnson & Johnson Plaza
(C) CITY: New Brunswick
(D) STATE: New Jersey
(E) COUNTRY: USA
(F) ZIP: 08933

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION: 435

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/589,958
(B) FILING DATE: 28-SEP-1990

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Stark, Michael
(B) REGISTRATION NUMBER: 32,495
(C) REFERENCE/DOCKET NUMBER: BCI-15

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 908-524-2817
(B) TELEFAX: 908-524-2808
(C) TELEX: 844-481

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 133 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn Cys
1               5                  10                  15

Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu Lys Gln Pro Pro Leu
            20                  25                  30

Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu
        35                  40                  45

Met Glu Asn Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala
    50                  55                  60

Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile Leu Lys Asn
65                  70                  75                  80

Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala Ala Pro Thr Arg His Pro
                85                  90                  95

Ile His Ile Lys Asp Gly Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr
            100                 105                 110

Phe Tyr Leu Lys Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu
        115                 120                 125

Ser Leu Ala Ile Phe
    130
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 79 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn Cys
1               5                  10                  15

Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu Lys Gln Pro Pro Leu
            20                  25                  30

Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu
        35                  40                  45

Met Glu Asn Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala
    50                  55                  60

Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile Leu Lys
65                  70                  75
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 166 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Ala Pro Pro Arg Leu Ile Cys Asp Ser Arg Val Leu Glu Arg Tyr Leu
1               5                  10                  15

Leu Glu Ala Lys Glu Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His
            20                  25                  30

Cys Ser Leu Asn Glu Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe
        35                  40                  45

Tyr Ala Trp Lys Arg Met Glu Val Gly Gln Gln Ala Val Glu Val Trp
    50                  55                  60

Gln Gly Leu Ala Leu Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu
65                  70                  75                  80

Leu Val Asn Ser Ser Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp
                85                  90                  95

Lys Ala Val Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu
            100                 105                 110
```

Gly Ala Gln Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala
115 120 125

Pro Leu Arg Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val
130 135 140

Tyr Ser Asn Phe Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala
145 150 155 160

Cys Arg Thr Gly Asp Arg
165

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 155 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Cys Asp Ser Arg Val Leu Glu Arg Tyr Leu Leu Glu Ala Lys Glu Ala
1 5 10 15

Glu Asn Ile Thr Thr Gly Cys Ala Glu His Cys Ser Leu Asn Glu Asn
20 25 30

Ile Thr Val Pro Asp Thr Lys Val Asn Phe Tyr Ala Trp Lys Arg Met
35 40 45

Glu Val Gly Gln Gln Ala Val Glu Val Trp Gln Gly Leu Ala Leu Leu
50 55 60

Ser Glu Ala Val Leu Arg Gly Gln Ala Leu Leu Val Asn Ser Ser Gln
65 70 75 80

Pro Trp Glu Pro Leu Gln Leu His Val Asp Lys Ala Val Ser Gly Leu
85 90 95

Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu Gly Ala Gln Lys Glu Ala
100 105 110

```
Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala Pro Leu Arg Thr Ile Thr
        115                 120                 125

Ala Asp Thr Phe Arg Lys Leu Phe Arg Val Tyr Ser Asn Phe Leu Arg
    130                 135                 140

Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala Cys
145                 150                 155
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 174 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
1               5                   10                  15

Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
            20                  25                  30

Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val
        35                  40                  45

Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys
    50                  55                  60

Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser
65                  70                  75                  80

Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser
                85                  90                  95

Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp
            100                 105                 110

Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro
        115                 120                 125

Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe
    130                 135                 140
```

Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe
145 150 155 160

Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
165 170

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 302 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn Cys
1 5 10 15

Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu Lys Gln Pro Pro Leu
20 25 30

Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu
35 40 45

Met Glu Asn Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala
50 55 60

Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile Leu Lys Asn
65 70 75 80

Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala Ala Pro Thr Arg His Pro
85 90 95

Ile His Ile Lys Asp Gly Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr
100 105 110

Phe Tyr Leu Lys Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu
115 120 125

Ser Leu Ala Ile Phe Leu Asp Met Ala Pro Pro Arg Leu Ile Cys Asp
130 135 140

Ser Arg Val Leu Glu Arg Tyr Leu Leu Glu Ala Lys Glu Ala Glu Asn
145 150 155 160

```
Ile Thr Thr Gly Cys Ala Glu His Cys Ser Leu Asn Glu Asn Ile Thr
                165                 170                 175

Val Pro Asp Thr Lys Val Asn Phe Tyr Ala Trp Lys Arg Met Glu Val
            180                 185                 190

Gly Gln Gln Ala Val Glu Val Trp Gln Gly Leu Ala Leu Leu Ser Glu
        195                 200                 205

Ala Val Leu Arg Gly Gln Ala Leu Leu Val Asn Ser Ser Gln Pro Trp
    210                 215                 220

Glu Pro Leu Gln Leu His Val Asp Lys Ala Val Ser Gly Leu Arg Ser
225                 230                 235                 240

Leu Thr Thr Leu Leu Arg Ala Leu Gly Ala Gln Lys Glu Ala Ile Ser
                245                 250                 255

Pro Pro Asp Ala Ala Ser Ala Ala Pro Leu Arg Thr Ile Thr Ala Asp
            260                 265                 270

Thr Phe Arg Lys Leu Phe Arg Val Tyr Ser Asn Phe Leu Arg Gly Lys
        275                 280                 285

Leu Lys Leu Tyr Thr Gly Glu Ala Cys Arg Thr Gly Asp Arg
    290                 295                 300
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 321 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn Cys
1               5                   10                  15

Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu Lys Gln Pro Pro Leu
            20                  25                  30

Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu
        35                  40                  45
```

Met Glu Asn Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala
50 55 60

Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile Leu Lys Asn
65 70 75 80

Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala Ala Pro Thr Arg His Pro
85 90 95

Ile His Ile Lys Asp Gly Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr
100 105 110

Phe Tyr Leu Lys Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu
115 120 125

Ser Leu Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
130 135 140

Gly Gly Ser Ala Leu Ala Ile Phe Leu Asp Met Ala Pro Pro Arg Leu
145 150 155 160

Ile Cys Asp Ser Arg Val Leu Glu Arg Tyr Leu Leu Glu Ala Lys Glu
165 170 175

Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His Cys Ser Leu Asn Glu
180 185 190

Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe Tyr Ala Trp Lys Arg
195 200 205

Met Glu Val Gly Gln Gln Ala Val Glu Val Trp Gln Gly Leu Ala Leu
210 215 220

Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu Leu Val Asn Ser Ser
225 230 235 240

Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp Lys Ala Val Ser Gly
245 250 255

Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu Gly Ala Gln Lys Glu
260 265 270

Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala Pro Leu Arg Thr Ile
275 280 285

Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val Tyr Ser Asn Phe Leu
290 295 300

Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala Cys Arg Thr Gly Asp
305 310 315 320

Arg

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 303 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Ala Pro Pro Arg Leu Ile Cys Asp Ser Arg Val Leu Glu Arg Tyr Leu
1               5                  10                  15

Leu Glu Ala Lys Glu Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His
            20                  25                  30

Cys Ser Leu Asn Glu Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe
        35                  40                  45

Tyr Ala Trp Lys Arg Met Glu Val Gly Gln Gln Ala Val Glu Val Trp
    50                  55                  60

Gln Gly Leu Ala Leu Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu
65                  70                  75                  80

Leu Val Asn Ser Ser Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp
                85                  90                  95

Lys Ala Val Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu
            100                 105                 110

Gly Ala Gln Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala
        115                 120                 125

Pro Leu Arg Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val
    130                 135                 140

Tyr Ser Asn Phe Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala
145                 150                 155                 160

Cys Arg Thr Gly Asp Arg Ala Ala Ala Met Ala Pro Met Thr Gln Thr
                165                 170                 175

Thr Ser Leu Lys Thr Ser Trp Val Asn Cys Ser Asn Met Ile Asp Glu
            180                 185                 190
```

Ile Ile Thr His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn
195 200 205

Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg
210 215 220

Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn
225 230 235 240

Ala Ser Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro
245 250 255

Leu Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
260 265 270

Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr Leu
275 280 285

Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe
290 295 300

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 322 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Ala Pro Pro Arg Leu Ile Cys Asp Ser Arg Val Leu Glu Arg Tyr Leu
1 5 10 15

Leu Glu Ala Lys Glu Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His
20 25 30

Cys Ser Leu Asn Glu Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe
35 40 45

Tyr Ala Trp Lys Arg Met Glu Val Gly Gln Gln Ala Val Glu Val Trp
50 55 60

Gln Gly Leu Ala Leu Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu
65 70 75 80

Leu Val Asn Ser Ser Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp
85 90 95

Lys Ala Val Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu
100 105 110

Gly Ala Gln Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala
115 120 125

Pro Leu Arg Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val
130 135 140

Tyr Ser Asn Phe Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala
145 150 155 160

Cys Arg Thr Gly Asp Arg Ala Ala Ala Ser Gly Gly Gly Gly Ser Gly
165 170 175

Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Ala Ala Met Ala Pro Met
180 185 190

Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn Cys Ser Asn Met
195 200 205

Ile Asp Glu Ile Ile Thr His Leu Lys Gln Pro Pro Leu Pro Leu Leu
210 215 220

Asp Phe Asn Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn
225 230 235 240

Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala Val Lys Ser
245 250 255

Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro
260 265 270

Cys Leu Pro Leu Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile
275 280 285

Lys Asp Gly Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu
290 295 300

Lys Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu Ser Leu Ala
305 310 315 320

Ile Phe

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 317 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn Cys
1 5 10 15

Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu Lys Gln Pro Pro Leu
20 25 30

Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu
35 40 45

Met Glu Asn Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala
50 55 60

Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile Leu Lys Asn
65 70 75 80

Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala Ala Pro Thr Arg His Pro
85 90 95

Ile His Ile Lys Asp Gly Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr
100 105 110

Phe Tyr Leu Lys Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu
115 120 125

Ser Leu Ala Ile Phe Leu Glu Ala Ala Ala Ser Leu Pro Ala Met Thr
130 135 140

Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Cys
145 150 155 160

Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln Glu
165 170 175

Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val Leu
180 185 190

Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro
195 200 205

Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser Gly
210 215 220

```
Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro
225                 230                 235                 240

Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp Phe
                245                 250                 255

Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro Ala
            260                 265                 270

Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln
        275                 280                 285

Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe Leu
    290                 295                 300

Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
305                 310                 315
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 994 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 14..977

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 71..977

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
AATTGCCGCC ACC ATG AGC CGC CTG CCC GTC CTG CTC CTG CTC CAA CTC          49
               Met Ser Arg Leu Pro Val Leu Leu Leu Leu Gln Leu
               -19             -15                 -10

CTG GTC CGC CCC GGA CTC CAA GCT CCC ATG ACC CAG ACA ACT AGT TTG          97
Leu Val Arg Pro Gly Leu Gln Ala Pro Met Thr Gln Thr Thr Ser Leu
        -5                  1               5
```

```
AAG ACA AGC TGG GTT AAC TGC TCT AAC ATG ATC GAT GAA ATT ATA ACA      145
Lys Thr Ser Trp Val Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr
 10                  15                  20                  25

CAC TTA AAC GAG CCA CCT TTG CCT TTG CTG GAC TTC AAC AAC CTC AAT      193
His Leu Asn Glu Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn
                 30                  35                  40

GGG GAA GAC CAA GAC ATT CTG ATG GAA AAT AAC CTT CGA AGG CCA AAC      241
Gly Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
             45                  50                  55

CTG GAG GCA TTC AAC AGG GCT GTC AAG AGT TTA CAG AAT GCA TCA GCA      289
Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser Ala
         60                  65                  70

ATT GAG AGC ATT CTT AAA AAT CTC CTG CCA TGT CTG CCC CTG GCC ACG      337
Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr
     75                  80                  85

GCC GCA CCC ACG CGA CAT CCA ATC CAT ATC AAG GAC GGT GAC TGG AAT      385
Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp Trp Asn
 90                  95                  100                 105

GAA TTC CGG AGG AAA CTG ACG TTC TAT CTG AAA ACC CTT GAG AAT GCG      433
Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr Leu Glu Asn Ala
                110                 115                 120

CAG GCT CAA CAG ACG ACT TTG TCG CTA GCG ATC TTT CTA GAC ATG GCC      481
Gln Ala Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe Leu Asp Met Ala
            125                 130                 135

CCA CCA CGC CTC ATC TGT GAC AGC CGA GTC CTG GAG AGG TAC CTC TTG      529
Pro Pro Arg Leu Ile Cys Asp Ser Arg Val Leu Glu Arg Tyr Leu Leu
        140                 145                 150

GAG GCC AAG GAG GCC GAG AAT ATC ACG ACG GGC TGT GCT GAA CAC TGC      577
Glu Ala Lys Glu Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His Cys
    155                 160                 165

AGC TTG AAT GAG AAT ATC ACT GTC CCA GAC ACC AAA GTT AAT TTC TAC      625
Ser Leu Asn Glu Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe Tyr
170                 175                 180                 185

GCG TGG AAG AGG ATG GAG GTC GGC CAG CAG GCC GTA GAA GTC TGG CAG      673
Ala Trp Lys Arg Met Glu Val Gly Gln Gln Ala Val Glu Val Trp Gln
                190                 195                 200

GGC CTG GCC CTG CTG TCG GAA GCT GTC CTG CGG GGC CAG GCC CTG TTG      721
Gly Leu Ala Leu Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu Leu
            205                 210                 215
```

```
GTC AAC TCG AGC CAG CCG TGG GAG CCC CTG CAA CTG CAT GTG GAT AAA      769
Val Asn Ser Ser Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp Lys
        220                 225                 230

GCC GTC AGT GGC CTT CGC AGC CTC ACC ACT CTG CTT CGG GCT CTG GGA      817
Ala Val Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu Gly
    235                 240                 245

GCT CAG AAG GAA GCC ATC TCC CCT CCA GAT GCG GCC TCA GCT GCT CCA      865
Ala Gln Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala Pro
250                 255                 260                 265

CTC CGA ACA ATC ACT GCT GAC ACT TTC CGC AAA CTC TTC CGA GTC TAC      913
Leu Arg Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val Tyr
                270                 275                 280

TCC AAT TTC CTC CGG GGA AAG CTG AAG CTG TAC ACA GGG GAG GCA TGC      961
Ser Asn Phe Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala Cys
            285                 290                 295

AGG ACA GGG GAC AGA T GATAAGGATC CGAATTC                            994
Arg Thr Gly Asp Arg
        300
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1015 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 8..998

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 89..998

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
TCGAGCC ATG GGG GTG CAC GAA TGT CCT GCC TGG CTG TGG CTT CTC CTG       49
        Met Gly Val His Glu Cys Pro Ala Trp Leu Trp Leu Leu Leu
        -27     -25                 -20                 -15
```

```
TCC CTG CTG TCG CTC CCT CTG GGC CTC CCA GTC CTG GGC GCC CCA CCA          97
Ser Leu Leu Ser Leu Pro Leu Gly Leu Pro Val Leu Gly Ala Pro Pro
            -10                 -5                  1

CGC CTC ATC TGT GAC AGC CGA GTC CTG GAG AGG TAC CTC TTG GAG GCC         145
Arg Leu Ile Cys Asp Ser Arg Val Leu Glu Arg Tyr Leu Leu Glu Ala
      5                 10                  15

AAG GAG GCC GAG AAT ATC ACG ACG GGC TGT GCT GAA CAC TGC AGC TTG         193
Lys Glu Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His Cys Ser Leu
 20                  25                  30                  35

AAT GAG AAT ATC ACT GTC CCA GAC ACC AAA GTT AAT TTC TAC GCG TGG         241
Asn Glu Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe Tyr Ala Trp
                40                  45                  50

AAG AGG ATG GAG GTC GGC CAG CAG GCC GTA GAA GTC TGG CAG GGC CTG         289
Lys Arg Met Glu Val Gly Gln Gln Ala Val Glu Val Trp Gln Gly Leu
            55                  60                  65

GCC CTG CTG TCG GAA GCT GTC CTG CGG GGC CAG GCC CTG TTG GTC AAC         337
Ala Leu Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu Leu Val Asn
        70                  75                  80

TCG AGC CAG CCG TGG GAG CCC CTG CAA CTG CAT GTG GAT AAA GCC GTC         385
Ser Ser Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp Lys Ala Val
    85                  90                  95

AGT GGC CTT CGC AGC CTC ACC ACT CTG CTT CGG GCT CTG GGA GCT CAG         433
Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu Gly Ala Gln
100                 105                 110                 115

AAG GAA GCC ATC TCC CCT CCA GAT GCG GCC TCA GCT GCT CCA CTC CGA         481
Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala Pro Leu Arg
                120                 125                 130

ACA ATC ACT GCT GAC ACT TTC CGC AAA CTC TTC CGA GTC TAC TCC AAT         529
Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val Tyr Ser Asn
            135                 140                 145

TTC CTC CGG GGA AAG CTG AAG CTG TAC ACA GGG GAG GCA TGC AGG ACA         577
Phe Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala Cys Arg Thr
        150                 155                 160

GGG GAC AGA GCG GCC GCC ATG GCT CCC ATG ACC CAG ACA ACT AGT TTG         625
Gly Asp Arg Ala Ala Ala Met Ala Pro Met Thr Gln Thr Thr Ser Leu
    165                 170                 175

AAG ACA AGC TGG GTT AAC TGC TCT AAC ATG ATC GAT GAA ATT ATA ACA         673
Lys Thr Ser Trp Val Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr
180                 185                 190                 195
```

```
CAC TTA AAC GAG CCA CCT TTG CCT TTG CTG GAC TTC AAC AAC CTC AAT      721
His Leu Asn Glu Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn
                200                 205                 210

GGG GAA GAC CAA GAC ATT CTG ATG GAA AAT AAC CTT CGA AGG CCA AAC      769
Gly Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
            215                 220                 225

CTG GAG GCA TTC AAC AGG GCT GTC AAG AGT TTA CAG AAT GCA TCA GCA      817
Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser Ala
        230                 235                 240

ATT GAG AGC ATT CTT AAA AAT CTC CTG CCA TGT CTG CCC CTG GCC ACG      865
Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr
    245                 250                 255

GCC GCA CCC ACG CGA CAT CCA ATC CAT ATC AAG GAC GGT GAC TGG AAT      913
Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp Trp Asn
260                 265                 270                 275

GAA TTC CGG AGG AAA CTG ACG TTC TAT CTG AAA ACC CTT GAG AAT GCG      961
Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr Leu Glu Asn Ala
                280                 285                 290

CAG GCT CAA CAG ACG ACT TTG TCG CTA GCG ATC TTT T AGTAAGGATC        1008
Gln Ala Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe
            295                 300

CGAATTC                                                            1015
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1039 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 14..1021

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 71..1021

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
AATTGCCGCC ACC ATG AGC CGC CTG CCC GTC CTG CTC CTG CTC CAA CTC          49
               Met Ser Arg Leu Pro Val Leu Leu Leu Leu Gln Leu
               -19             -15                 -10

CTG GTC CGC CCC GGA CTC CAA GCT CCC ATG ACC CAG ACA ACT AGT TTG          97
Leu Val Arg Pro Gly Leu Gln Ala Pro Met Thr Gln Thr Thr Ser Leu
         -5                  1               5

AAG ACA AGC TGG GTT AAC TGC TCT AAC ATG ATC GAT GAA ATT ATA ACA         145
Lys Thr Ser Trp Val Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr
 10                  15                  20                  25

CAC TTA AAC GAG CCA CCT TTG CCT TTG CTG GAC TTC AAC AAC CTC AAT         193
His Leu Asn Glu Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn
                 30                  35                  40

GGG GAA GAC CAA GAC ATT CTG ATG GAA AAT AAC CTT CGA AGG CCA AAC         241
Gly Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
             45                  50                  55

CTG GAG GCA TTC AAC AGG GCT GTC AAG AGT TTA CAG AAT GCA TCA GCA         289
Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser Ala
         60                  65                  70

ATT GAG AGC ATT CTT AAA AAT CTC CTG CCA TGT CTG CCC CTG GCC ACG         337
Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr
     75                  80                  85

GCC GCA CCC ACG CGA CAT CCA ATC CAT ATC AAG GAC GGT GAC TGG AAT         385
Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp Trp Asn
 90                  95                  100                 105

GAA TTC CGG AGG AAA CTG ACG TTC TAT CTG AAA ACC CTT GAG AAT GCG         433
Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr Leu Glu Asn Ala
                110                 115                 120

CAG GCT CAA CAG ACG ACT TTG TCG CTA GCG ATC TTT CTA GAA GCG GCC         481
Gln Ala Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe Leu Glu Ala Ala
            125                 130                 135

GCA AGC TTA CCT GCC ATG ACC CCC CTG GGC CCT GCC AGC TCC CTG CCC         529
Ala Ser Leu Pro Ala Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro
        140                 145                 150

CAG AGC TTC CTG CTC AAG TGC TTA GAG CAA GTG AGG AAG ATC CAG GGC         577
Gln Ser Phe Leu Leu Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly
    155                 160                 165

GAT GGC GCA GCG CTC CAG GAG AAG CTG TGT GCC ACC TAC AAG CTG TGC         625
Asp Gly Ala Ala Leu Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys
170                 175                 180                 185
```

```
CAC CCC GAG GAG CTG GTG CTG CTC GGA CAC TCT CTG GGC ATC CCC TGG      673
His Pro Glu Glu Leu Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp
                190                 195                 200

GCT CCC CTG AGC TCC TGC CCC AGC CAG GCC CTG CAG CTG GCA GGC TGC      721
Ala Pro Leu Ser Ser Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys
            205                 210                 215

TTG AGC CAA CTC CAT AGC GGC CTT TTC CTC TAC CAG GGG CTC CTG CAG      769
Leu Ser Gln Leu His Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln
        220                 225                 230

GCC CTG GAA GGG ATA TCC CCC GAG TTG GGT CCC ACC TTG CAC ACA CTG      817
Ala Leu Glu Gly Ile Ser Pro Glu Leu Gly Pro Thr Leu His Thr Leu
    235                 240                 245

CAG CTG GAC GTC GCC GAC TTT GCC ACC ACC ATC TGG CAG CAG ATG GAA      865
Gln Leu Asp Val Ala Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu
250                 255                 260                 265

GAA CTG GGA ATG GCC CCT GCC CTG CAG CCC ACC CAG GGT GCC ATG CCG      913
Glu Leu Gly Met Ala Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro
                270                 275                 280

GCC TTC GCC TCT GCT TTC CAG CGC CGG GCA GGA GGG GTC CTG GTT GCT      961
Ala Phe Ala Ser Ala Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala
            285                 290                 295

AGC CAT CTG CAG AGC TTC CTG GAG GTG TCG TAC CGC GTT CTA CGC CAC     1009
Ser His Leu Gln Ser Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His
        300                 305                 310

CTT GCG CAG CCC TGATAAGGAT CCGAATTC                                 1039
Leu Ala Gln Pro
    315
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1051 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 14..1033

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 71..1033

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
AATTGCCGCC ACC ATG AGC CGC CTG CCC GTC CTG CTC CTG CTC CAA CTC          49
               Met Ser Arg Leu Pro Val Leu Leu Leu Leu Gln Leu
               -19             -15                 -10

CTG GTC CGC CCC GGA CTC CAA GCT CCC ATG ACC CAG ACA ACT AGT TTG          97
Leu Val Arg Pro Gly Leu Gln Ala Pro Met Thr Gln Thr Thr Ser Leu
         -5                  1               5

AAG ACA AGC TGG GTT AAC TGC TCT AAC ATG ATC GAT GAA ATT ATA ACA         145
Lys Thr Ser Trp Val Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr
 10                  15                  20                  25

CAC TTA AAC GAG CCA CCT TTG CCT TTG CTG GAC TTC AAC AAC CTC AAT         193
His Leu Asn Glu Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn
                 30                  35                  40

GGG GAA GAC CAA GAC ATT CTG ATG GAA AAT AAC CTT CGA AGG CCA AAC         241
Gly Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
             45                  50                  55

CTG GAG GCA TTC AAC AGG GCT GTC AAG AGT TTA CAG AAT GCA TCA GCA         289
Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser Ala
         60                  65                  70

ATT GAG AGC ATT CTT AAA AAT CTC CTG CCA TGT CTG CCC CTG GCC ACG         337
Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr
     75                  80                  85

GCC GCA CCC ACG CGA CAT CCA ATC CAT ATC AAG GAC GGT GAC TGG AAT         385
Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp Trp Asn
 90                  95                  100                 105

GAA TTC CGG AGG AAA CTG ACG TTC TAT CTG AAA ACC CTT GAG AAT GCG         433
Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr Leu Glu Asn Ala
                110                 115                 120

CAG GCT CAA CAG ACG ACT TTG TCG CTA GCG TCC GGA GGC GGT GGC TCG         481
Gln Ala Gln Gln Thr Thr Leu Ser Leu Ala Ser Gly Gly Gly Gly Ser
            125                 130                 135

GGC GGT GGC GGC TCG GGT GGC GGC GGC TCT GCG CTA GCG ATC TTT CTA         529
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Leu Ala Ile Phe Leu
        140                 145                 150
```

```
GAC ATG GCC CCA CCA CGC CTC ATC TGT GAC AGC CGA GTC CTG GAG AGG        577
Asp Met Ala Pro Pro Arg Leu Ile Cys Asp Ser Arg Val Leu Glu Arg
    155                 160                 165

TAC CTC TTG GAG GCC AAG GAG GCC GAG AAT ATC ACG ACG GGC TGT GCT        625
Tyr Leu Leu Glu Ala Lys Glu Ala Glu Asn Ile Thr Thr Gly Cys Ala
170                 175                 180                 185

GAA CAC TGC AGC TTG AAT GAG AAT ATC ACT GTC CCA GAC ACC AAA GTT        673
Glu His Cys Ser Leu Asn Glu Asn Ile Thr Val Pro Asp Thr Lys Val
                190                 195                 200

AAT TTC TAC GCG TGG AAG AGG ATG GAG GTC GGC CAG CAG GCC GTA GAA        721
Asn Phe Tyr Ala Trp Lys Arg Met Glu Val Gly Gln Gln Ala Val Glu
            205                 210                 215

GTC TGG CAG GGC CTG GCC CTG CTG TCG GAA GCT GTC CTG CGG GGC CAG        769
Val Trp Gln Gly Leu Ala Leu Leu Ser Glu Ala Val Leu Arg Gly Gln
        220                 225                 230

GCC CTG TTG GTC AAC TCG AGC CAG CCG TGG GAG CCC CTG CAA CTG CAT        817
Ala Leu Leu Val Asn Ser Ser Gln Pro Trp Glu Pro Leu Gln Leu His
    235                 240                 245

GTG GAT AAA GCC GTC AGT GGC CTT CGC AGC CTC ACC ACT CTG CTT CGG        865
Val Asp Lys Ala Val Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu Arg
250                 255                 260                 265

GCT CTG GGA GCT CAG AAG GAA GCC ATC TCC CCT CCA GAT GCG GCC TCA        913
Ala Leu Gly Ala Gln Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser
                270                 275                 280

GCT GCT CCA CTC CGA ACA ATC ACT GCT GAC ACT TTC CGC AAA CTC TTC        961
Ala Ala Pro Leu Arg Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe
            285                 290                 295

CGA GTC TAC TCC AAT TTC CTC CGG GGA AAG CTG AAG CTG TAC ACA GGG       1009
Arg Val Tyr Ser Asn Phe Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly
        300                 305                 310

GAG GCA TGC AGG ACA GGG GAC AGA TGATAAGGAT CCGAATTC                   1051
Glu Ala Cys Arg Thr Gly Asp Arg
    315                 320
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1072 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 8..1054

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 89..1054

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
TCGAGCC ATG GGG GTG CAC GAA TGT CCT GCC TGG CTG TGG CTT CTC CTG          49
        Met Gly Val His Glu Cys Pro Ala Trp Leu Trp Leu Leu Leu
        -27     -25                 -20                 -15

TCC CTG CTG TCG CTC CCT CTG GGC CTC CCA GTC CTG GGC GCC CCA CCA          97
Ser Leu Leu Ser Leu Pro Leu Gly Leu Pro Val Leu Gly Ala Pro Pro
            -10                 -5                  1

CGC CTC ATC TGT GAC AGC CGA GTC CTG GAG AGG TAC CTC TTG GAG GCC         145
Arg Leu Ile Cys Asp Ser Arg Val Leu Glu Arg Tyr Leu Leu Glu Ala
      5                 10                  15

AAG GAG GCC GAG AAT ATC ACG ACG GGC TGT GCT GAA CAC TGC AGC TTG         193
Lys Glu Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His Cys Ser Leu
 20                 25                  30                  35

AAT GAG AAT ATC ACT GTC CCA GAC ACC AAA GTT AAT TTC TAC GCG TGG         241
Asn Glu Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe Tyr Ala Trp
                40                  45                  50

AAG AGG ATG GAG GTC GGC CAG CAG GCC GTA GAA GTC TGG CAG GGC CTG         289
Lys Arg Met Glu Val Gly Gln Gln Ala Val Glu Val Trp Gln Gly Leu
            55                  60                  65

GCC CTG CTG TCG GAA GCT GTC CTG CGG GGC CAG GCC CTG TTG GTC AAC         337
Ala Leu Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu Leu Val Asn
        70                  75                  80

TCG AGC CAG CCG TGG GAG CCC CTG CAA CTG CAT GTG GAT AAA GCC GTC         385
Ser Ser Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp Lys Ala Val
    85                  90                  95

AGT GGC CTT CGC AGC CTC ACC ACT CTG CTT CGG GCT CTG GGA GCT CAG         433
Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu Gly Ala Gln
100                 105                 110                 115
```

```
AAG GAA GCC ATC TCC CCT CCA GAT GCG GCC TCA GCT GCT CCA CTC CGA      481
Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala Pro Leu Arg
                120                 125                 130

ACA ATC ACT GCT GAC ACT TTC CGC AAA CTC TTC CGA GTC TAC TCC AAT      529
Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val Tyr Ser Asn
            135                 140                 145

TTC CTC CGG GGA AAG CTG AAG CTG TAC ACA GGG GAG GCA TGC AGG ACA      577
Phe Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala Cys Arg Thr
        150                 155                 160

GGG GAC AGA GCG GCC GCC TCC GGA GGC GGT GGC TCG GGC GGT GGC GGC      625
Gly Asp Arg Ala Ala Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
    165                 170                 175

TCG GGT GGC GGC GGC TCT GCG GCC GCC ATG GCT CCC ATG ACC CAG ACA      673
Ser Gly Gly Gly Gly Ser Ala Ala Ala Met Ala Pro Met Thr Gln Thr
180                 185                 190                 195

ACT AGT TTG AAG ACA AGC TGG GTT AAC TGC TCT AAC ATG ATC GAT GAA      721
Thr Ser Leu Lys Thr Ser Trp Val Asn Cys Ser Asn Met Ile Asp Glu
                200                 205                 210

ATT ATA ACA CAC TTA AAC GAG CCA CCT TTG CCT TTG CTG GAC TTC AAC      769
Ile Ile Thr His Leu Asn Glu Pro Pro Leu Pro Leu Leu Asp Phe Asn
            215                 220                 225

AAC CTC AAT GGG GAA GAC CAA GAC ATT CTG ATG GAA AAT AAC CTT CGA      817
Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg
        230                 235                 240

AGG CCA AAC CTG GAG GCA TTC AAC AGG GCT GTC AAG AGT TTA CAG AAT      865
Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn
    245                 250                 255

GCA TCA GCA ATT GAG AGC ATT CTT AAA AAT CTC CTG CCA TGT CTG CCC      913
Ala Ser Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro
260                 265                 270                 275

CTG GCC ACG GCC GCA CCC ACG CGA CAT CCA ATC CAT ATC AAG GAC GGT      961
Leu Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
                280                 285                 290

GAC TGG AAT GAA TTC CGG AGG AAA CTG ACG TTC TAT CTG AAA ACC CTT     1009
Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr Leu
            295                 300                 305

GAG AAT GCG CAG GCT CAA CAG ACG ACT TTG TCG CTA GCG ATC TTT         1054
Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe
        310                 315                 320

TAGTAAGGAT CCGAATTC                                                1072
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 429 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 10..411

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 13..411

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
AAGCTTACC ATG GCT CCC ATG ACC CAG ACA ACT AGT TTG AAG ACA AGC          48
          Met Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser
           -1   1               5                  10

TGG GTT AAC TGC TCT AAC ATG ATC GAT GAA ATT ATA ACA CAC TTA AAC         96
Trp Val Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu Asn
        15                  20                  25

GAG CCA CCT TTG CCT TTG CTG GAC TTC AAC AAC CTC AAT GGG GAA GAC        144
Glu Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly Glu Asp
     30                  35                  40

CAA GAC ATT CTG ATG GAA AAT AAC CTT CGA AGG CCA AAC CTG GAG GCA        192
Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn Leu Glu Ala
 45                  50                  55                  60

TTC AAC AGG GCT GTC AAG AGT TTA CAG AAT GCA TCA GCA ATT GAG AGC        240
Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser
                 65                  70                  75

ATT CTT AAA AAT CTC CTG CCA TGT CTG CCC CTG GCC ACG GCC GCA CCC        288
Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala Ala Pro
             80                  85                  90

ACG CGA CAT CCA ATC CAT ATC AAG GAC GGT GAC TGG AAT GAA TTC CGG        336
Thr Arg His Pro Ile His Ile Lys Asp Gly Asp Trp Asn Glu Phe Arg
        95                  100                 105
```

```
AGG AAA CTG ACG TTC TAT CTG AAA ACC CTT GAG AAT GCG CAG GCT CAA      384
Arg Lys Leu Thr Phe Tyr Leu Lys Thr Leu Glu Asn Ala Gln Ala Gln
    110                 115                 120

CAG ACG ACT TTG TCG CTA GCG ATC TTT TAGTAAGGAT CCGAATTC             429
Gln Thr Thr Leu Ser Leu Ala Ile Phe
125                 130
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 532 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 14..514

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 17..514

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
AAGCTTACCT GCC ATG GCC CCA CCA CGC CTC ATC TGT GAC AGC CGA GTC      49
               Met Ala Pro Pro Arg Leu Ile Cys Asp Ser Arg Val
                -1   1               5                  10

CTG GAG AGG TAC CTC TTG GAG GCC AAG GAG GCC GAG AAT ATC ACG ACG     97
Leu Glu Arg Tyr Leu Leu Glu Ala Lys Glu Ala Glu Asn Ile Thr Thr
            15                  20                  25

GGC TGT GCT GAA CAC TGC AGC TTG AAT GAG AAT ATC ACT GTC CCA GAC     145
Gly Cys Ala Glu His Cys Ser Leu Asn Glu Asn Ile Thr Val Pro Asp
        30                  35                  40

ACC AAA GTT AAT TTC TAC GCG TGG AAG AGG ATG GAG GTC GGC CAG CAG     193
Thr Lys Val Asn Phe Tyr Ala Trp Lys Arg Met Glu Val Gly Gln Gln
    45                  50                  55

GCC GTA GAA GTC TGG CAG GGC CTG GCC CTG CTG TCG GAA GCT GTC CTG     241
Ala Val Glu Val Trp Gln Gly Leu Ala Leu Leu Ser Glu Ala Val Leu
60                  65                  70                  75
```

```
CGG GGC CAG GCC CTG TTG GTC AAC TCG AGC CAG CCG TGG GAG CCC CTG        289
Arg Gly Gln Ala Leu Leu Val Asn Ser Ser Gln Pro Trp Glu Pro Leu
                80                  85                  90

CAA CTG CAT GTG GAT AAA GCC GTC AGT GGC CTT CGC AGC CTC ACC ACT        337
Gln Leu His Val Asp Lys Ala Val Ser Gly Leu Arg Ser Leu Thr Thr
            95                  100                 105

CTG CTT CGG GCT CTG GGA GCT CAG AAG GAA GCC ATC TCC CCT CCA GAT        385
Leu Leu Arg Ala Leu Gly Ala Gln Lys Glu Ala Ile Ser Pro Pro Asp
        110                 115                 120

GCG GCC TCA GCT GCT CCA CTC CGA ACA ATC ACT GCT GAC ACT TTC CGC        433
Ala Ala Ser Ala Ala Pro Leu Arg Thr Ile Thr Ala Asp Thr Phe Arg
    125                 130                 135

AAA CTC TTC CGA GTC TAC TCC AAT TTC CTC CGG GGA AAG CTG AAG CTG        481
Lys Leu Phe Arg Val Tyr Ser Asn Phe Leu Arg Gly Lys Leu Lys Leu
140                 145                 150                 155

TAC ACA GGG GAG GCA TGC AGG ACA GGG GAC AG ATGATAAGGA TCCGAATTC        532
Tyr Thr Gly Glu Ala Cys Arg Thr Gly Asp
                160                 165
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 556 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 14..538

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 17..538

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
AAGCTTACCT GCC ATG ACC CCC CTG GGC CCT GCC AGC TCC CTG CCC CAG         49
               Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln
                -1   1              5                  10
```

```
AGC TTC CTG CTC AAG TGC TTA GAG CAA GTG AGG AAG ATC CAG GGC GAT        97
Ser Phe Leu Leu Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp
               15                  20                  25

GGC GCA GCG CTC CAG GAG AAG CTG TGT GCC ACC TAC AAG CTG TGC CAC       145
Gly Ala Ala Leu Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His
          30                  35                  40

CCC GAG GAG CTG GTG CTG CTC GGA CAC TCT CTG GGC ATC CCC TGG GCT       193
Pro Glu Glu Leu Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala
     45                  50                  55

CCC CTG AGC TCC TGC CCC AGC CAG GCC CTG CAG CTG GCA GGC TGC TTG       241
Pro Leu Ser Ser Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu
 60                  65                  70                  75

AGC CAA CTC CAT AGC GGC CTT TTC CTC TAC CAG GGG CTC CTG CAG GCC       289
Ser Gln Leu His Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala
                 80                  85                  90

CTG GAA GGG ATA TCC CCC GAG TTG GGT CCC ACC TTG CAC ACA CTG CAG       337
Leu Glu Gly Ile Ser Pro Glu Leu Gly Pro Thr Leu His Thr Leu Gln
             95                  100                 105

CTG GAC GTC GCC GAC TTT GCC ACC ACC ATC TGG CAG CAG ATG GAA GAA       385
Leu Asp Val Ala Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu
        110                 115                 120

CTG GGA ATG GCC CCT GCC CTG CAG CCC ACC CAG GGT GCC ATG CCG GCC       433
Leu Gly Met Ala Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala
    125                 130                 135

TTC GCC TCT GCT TTC CAG CGC CGG GCA GGA GGG GTC CTG GTT GCT AGC       481
Phe Ala Ser Ala Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser
140                 145                 150                 155

CAT CTG CAG AGC TTC CTG GAG GTG TCG TAC CGC GTT CTA CGC CAC CTT       529
His Leu Gln Ser Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu
                160                 165                 170

GCG CAG CCC TGATAAGGAT CCGAATTC                                       556
Ala Gln Pro
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

AATTGCCGCC ACCATGAGCC GCCTGCCCGT CCTGCTCCT 39

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GCTCCAACTC CTGGTCCGCC CCGGACTCCA AGCTCCCATG ACCCAGACAA 50

(2) INFORMATION FOR SEQ ID NO:21.

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CTAGTTGTCT GGGTCATGGG AGCTTGGAGT CCGGGGCGG 39

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

ACCAGGAGTT GGAGCAGGAG CAGGACGGGC AGGCGGCTCA TGGTGGCGGC 50

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CTAGCGATCT TTCTAGA 17

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CATGTCTAGA AAGATCG 17

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CTAGCGTCCG GAGGCGGTGG CTCGGGCGGT GGCGGCTCGG GTGGCGGCGG CTCTGCG 57

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CTAGCGCAGA GCCGCCGCCA CCGCAGCCGC CACCGCCCGA GCCACCGCCT CCGGACG 57

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

TTGTCGCTAG CGTCCGGAGG C 21

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CTAGAAGCGG CCGCA 15

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

TTCGCCGGCG TTCGA 15

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TCGAGCCATG GGGGTGCACG AATGTCCT 28

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GCCTGGCTGT GGCTTCTCCT GTCCCTGCTG TC 32

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GCTCCCTCTG GGCCTCCCAG TCCTGGGCTG CA 32

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GCCCAGGACT GGGAGGCCCA GAGGGA 26

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GCGACAGCAG GGACAGGAGA AGCCACAGCC AGGCAGGACA TT 42

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

CGTGCACCCC CATGGC 16

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GCCCCACCAC GCCTCATCTG T 21

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GAATTCGGAT CCTTATCATC T 21

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CTAGTCTCTA GAATGGGGGT CCACGAATGT 30

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

AGCCATGGCG GCCGCTCTGT CCCCTGTCCT 30

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

GACAGAGCGG CCGCCATGGC TCCCATGACC 30

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

GAATTCGGAT CCTTACTAAA AGATCGCTAG 30

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GGCCGCTTCC GGAGGCGGTG GCTCGGGCGG TGGCGGCTCG GGTGGCGGCG GCTCTGC 57

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

GGCCGCAGAG CCGCCGCCAC CCGAGCCGCC ACCGCCCGAG CCACCGCCTC CGGCAGC 57

What is claimed is:

1. A recombinant hematopoietic molecule comprising at least a portion of a first hematopoietic molecule having early myeloid differentiation activity and at least a portion of a second hematopoietic molecule having late myeloid differentiation activity, said recombinant hematopoietic molecule having early myeloid differentiation activity associated with said first hematopoietic molecule and late myeloid differentiation activity associated with said second hematopoietic molecule.

2. A recombinant hematopoietic molecule of claim 1 wherein the first hematopoietic molecule is selected from the group consisting of IL-3 and GM-CSF.

3. A recombinant hematopoietic molecule of claim 1 wherein the second hematopoietic molecule is selected from the group consisting of Epo, G-CSF, IL-5 and M-CSF.

4. A recombinant hematopoietic molecule of claim 1 wherein the portion of the first hematopoietic molecule is linked to the portion of the second hematopoietic molecule by an amino acid linker sequence of at least two amino acid residues.

5. A recombinant hematopoietic molecule of claim 1 comprising SEQ ID NO: 1.

6. A recombinant hematopoietic molecule of claim 1 comprising an amino acid sequence contained within SEQ ID NO: 2.

7. A recombinant hematopoietic molecule of claim 1 comprising SEQ ID NO: 3.

8. A recombinant hematopoietic molecule of claim 1 comprising an amino acid sequence contained within SEQ ID NO: 4.

9. A recombinant hematopoietic molecule of claim 1 comprising SEQ ID NO: 5.

10. A recombinant hematopoietic molecule of claim 1 wherein the first hematopoietic molecule is IL-3 and the second hematopoietic molecule is Epo.

11. A recombinant hematopoietic molecule of claim 10 wherein the first hematopoietic molecule comprises the amino portion and the second hematopoietic molecule comprises the carboxy portion of the recombinant hematopoietic molecule.

12. A recombinant hematopoietic molecule of claim 11 which comprises SEQ ID NO: 6.

13. A recombinant hematopoietic molecule of claim 11 which comprises SEQ ID NO: 7.

14. A recombinant hematopoietic molecule of claim 10 wherein the first hematopoietic molecule comprises the carboxy portion and the second hematopoietic molecule comprises the amino portion of the recombinant hematopoietic molecule.

15. A recombinant hematopoietic molecule of claim 14 which comprises SEQ ID NO: 8.

16. A recombinant hematopoietic molecule of claim 14 which comprises SEQ ID NO: 9.

17. A recombinant hematopoietic molecule of claim 1 wherein the first hematopoietic molecule is IL-3 and the second hematopoietic molecule is G-CSF

18. A recombinant hematopoietic molecule of claim 17 wherein the first hematopoietic molecule comprises the amino portion and the second hematopoietic molecule comprises the carboxy portion of the recombinant hematopoietic molecule.

19. A recombinant hematopoietic molecule of claim 18 which comprises SEQ ID NO: 10.

20. A nucleic acid molecule which encodes the recombinant hematopoietic molecule of claim 1.

21. An expression vector which comprises the nucleic acid molecule of claim 20.

22. A host cell transformed with the expression vector of claim 19.

23. A host cell of claim 22 which comprises a mammalian cell.

24. A method for producing a recombinant hematopoietic molecule comprising at least a portion of a first hematopoietic molecule having early myeloid differentiation activity and at least a portion of a second hematopoietic molecule having late myeloid differentiation activity, which comprises culturing a host cell of claim 22 under suitable conditions so as to allow the expression of such recombinant hematopoietic molecule, and recovering such recombinant hematopoietic molecule.

25. A pharmaceutical composition which comprises a recombinant hematopoietic molecule of claim 1 and a pharmaceutically acceptable carrier.

26. A method for promoting hematopoiesis in a patient which comprises administering to such patient a pharmaceutical composition of claim 25.

FIG-1

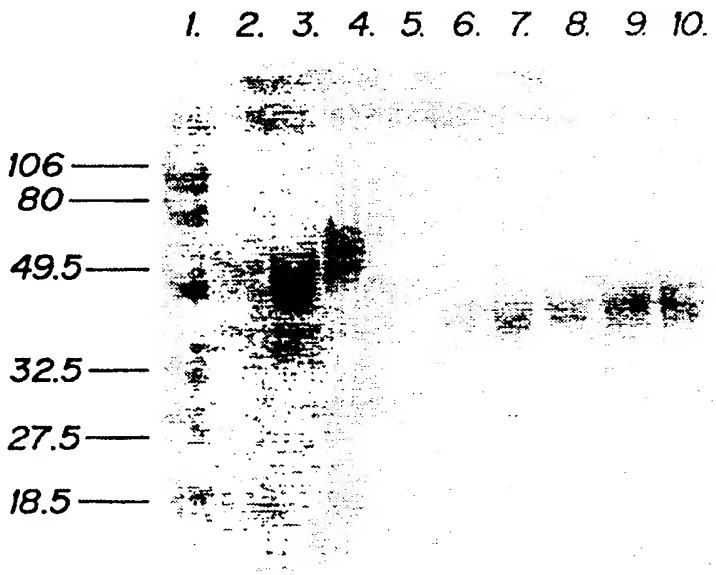


FIG-2

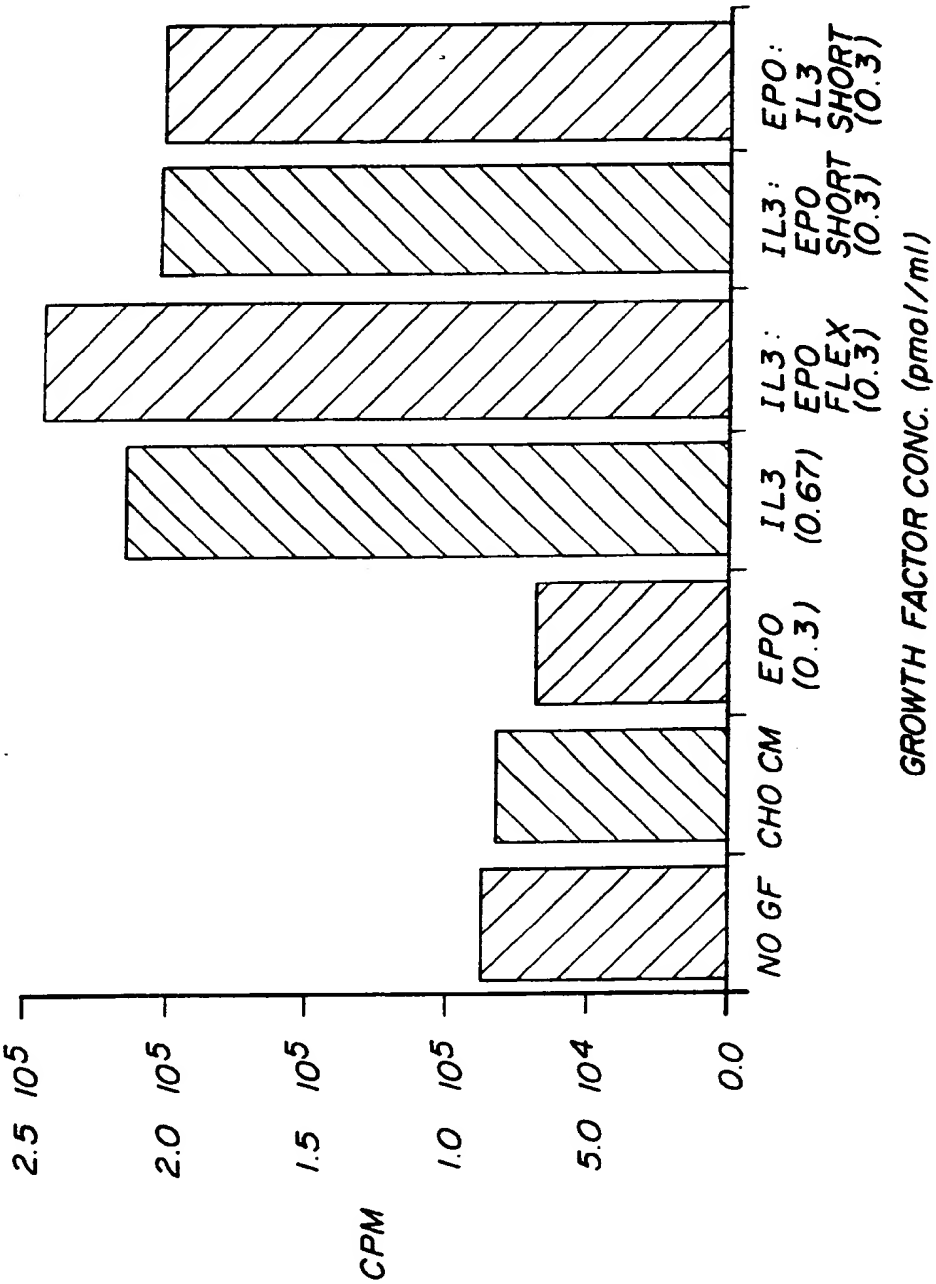

CPM
2.5 10^5
2.0 10^5
1.5 10^5
1.0 10^5
5.0 10^4
0.0
NO GF
CHO CM
EPO (0.3)
IL3 (0.67)
IL3: EPO FLEX (0.3)
IL3: EPO SHORT (0.3)
EPO: IL3 SHORT (0.3)
GROWTH FACTOR CONC. (pmol/ml)

FIG-3

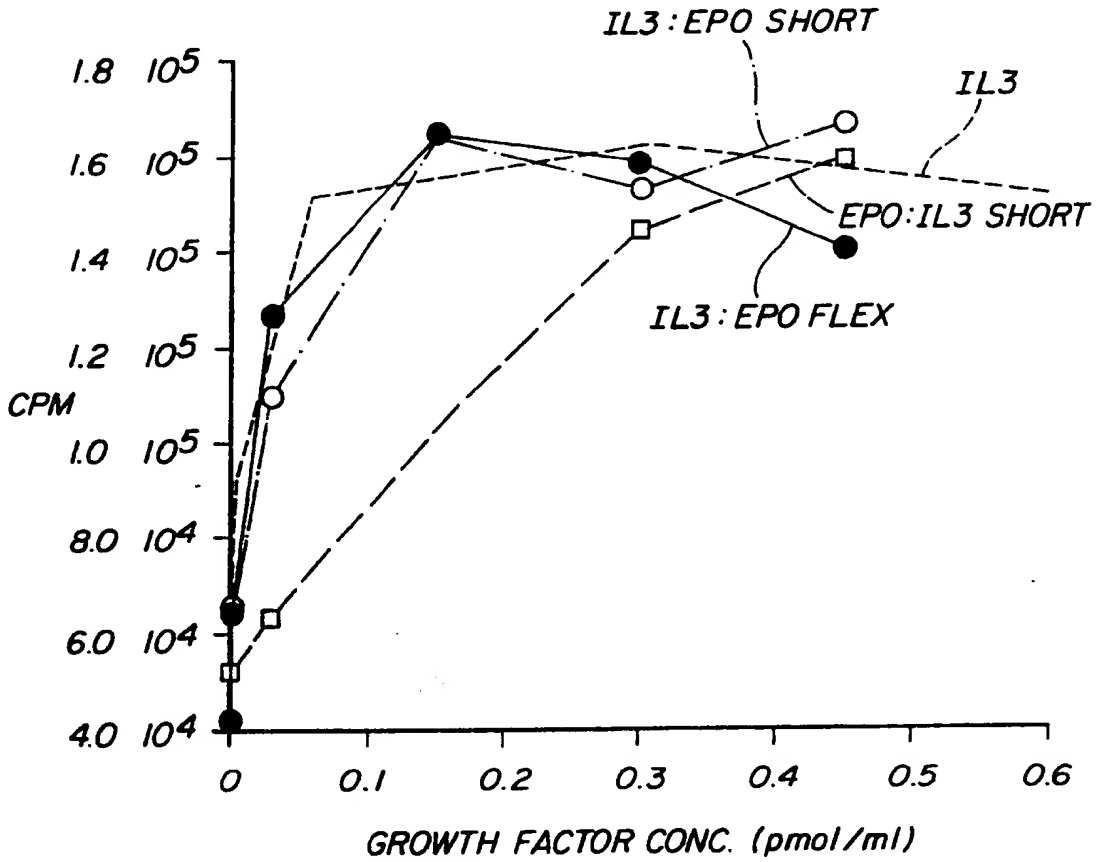

IL3 : EPO SHORT
IL3
EPO : IL3 SHORT
IL3 : EPO FLEX
1.8 10^5
1.6 10^5
1.4 10^5
1.2 10^5
CPM
1.0 10^5
8.0 10^4
6.0 10^4
4.0 10^4
0
0.1
0.2
0.3
0.4
0.5
0.6
GROWTH FACTOR CONC. (pmol/ml)

FIG-4

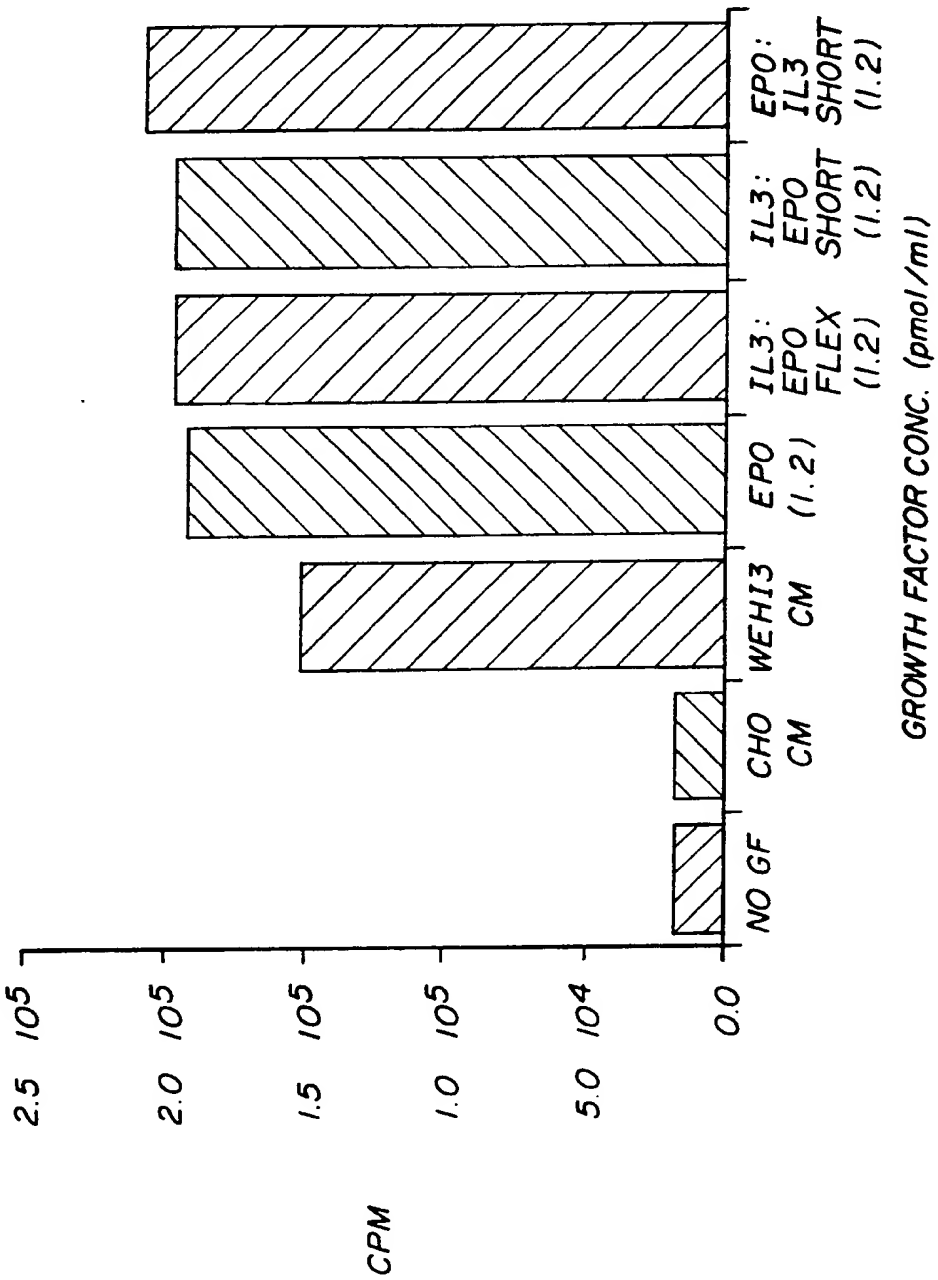

CPM
2.5 10^5
2.0 10^5
1.5 10^5
1.0 10^5
5.0 10^4
0.0
NO GF
CHO CM
WEHI3 CM
EPO (1.2)
IL3: EPO FLEX (1.2)
IL3: EPO SHORT (1.2)
EPO: IL3 SHORT (1.2)
GROWTH FACTOR CONC. (pmol/ml)

FIG-5

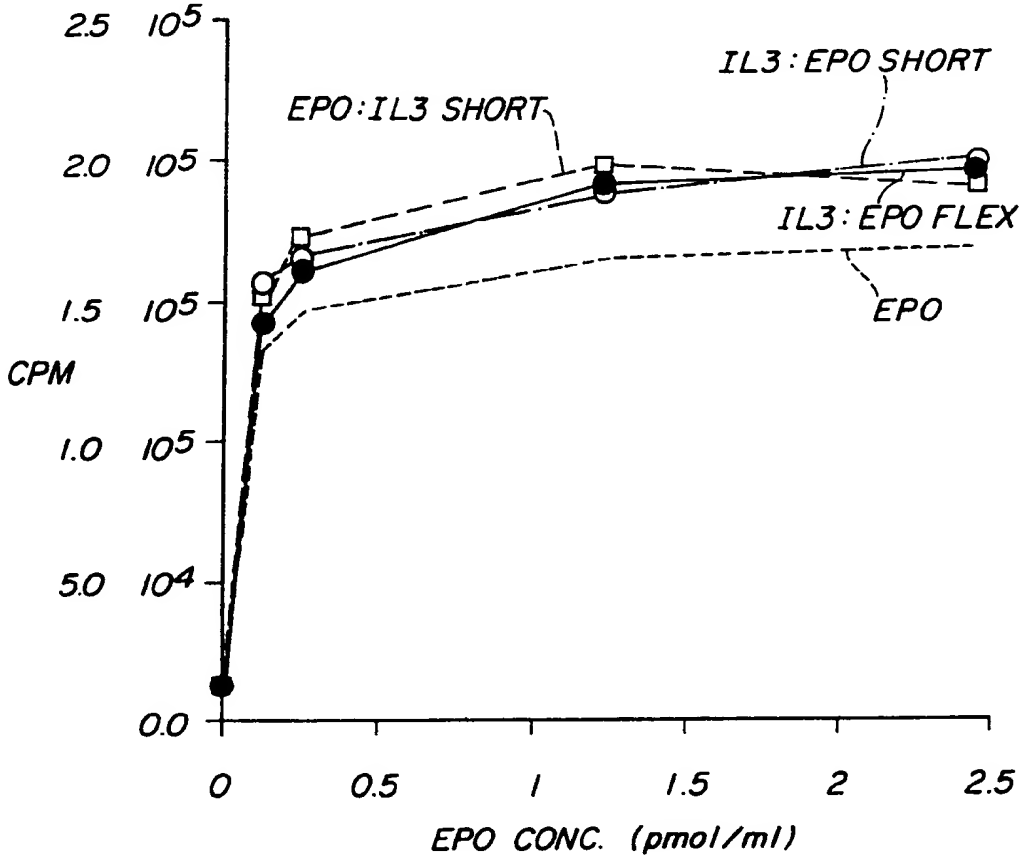

2.5 10^5
2.0 10^5
1.5 10^5
CPM
1.0 10^5
5.0 10^4
0.0
IL3:EPO SHORT
EPO:IL3 SHORT
IL3:EPO FLEX
EPO
0
0.5
1
1.5
2
2.5
EPO CONC. (pmol/ml)

FIG-6

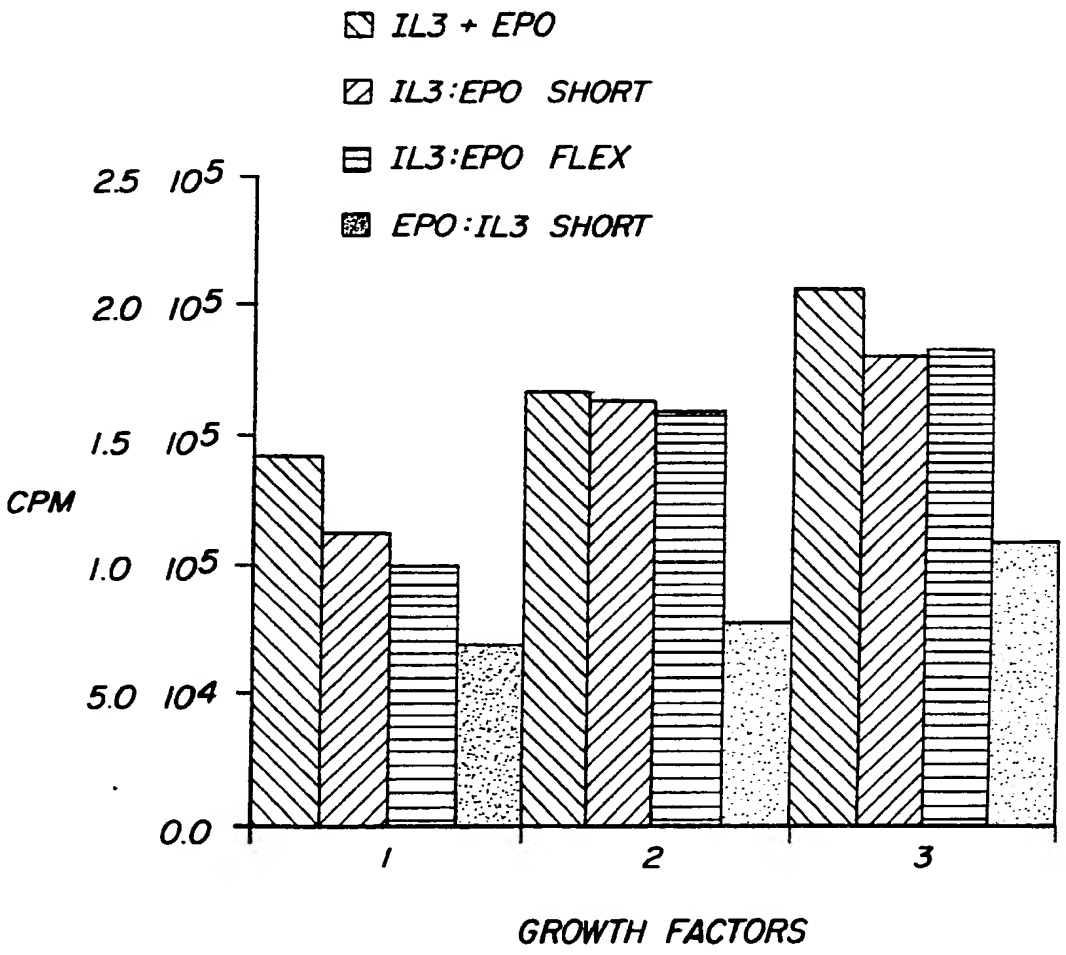

IL3 + EPO
IL3:EPO SHORT
IL3:EPO FLEX
EPO:IL3 SHORT
2.5 10^5
2.0 10^5
1.5 10^5
1.0 10^5
5.0 10^4
0.0
CPM
1
2
3
GROWTH FACTORS

FIG-7A

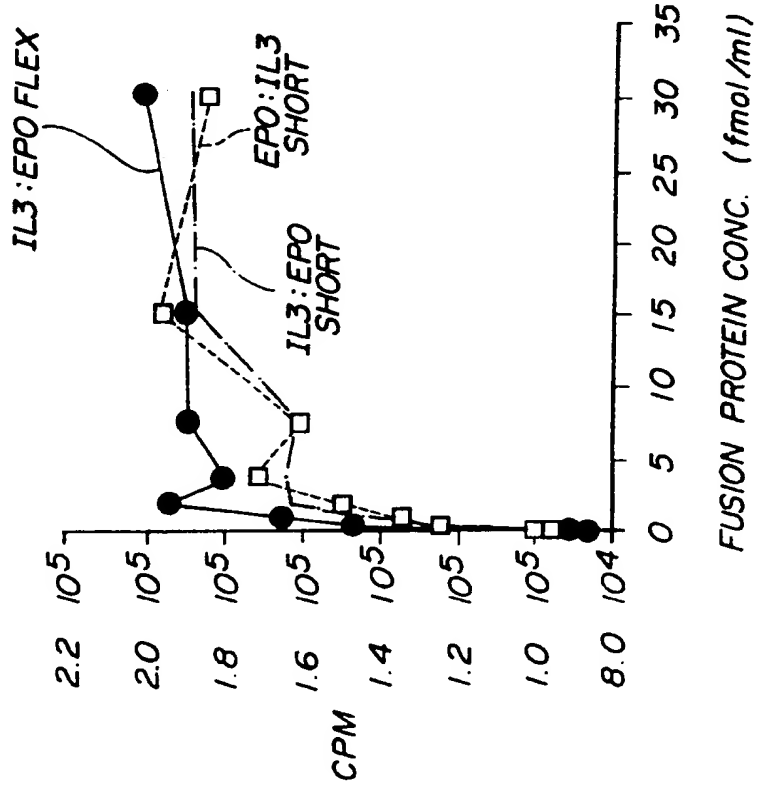

IL3:EPO FLEX
EPO:IL3 SHORT
IL3:EPO SHORT
CPM
2.2 10^5
2.0 10^5
1.8 10^5
1.6 10^5
1.4 10^5
1.2 10^5
1.0 10^5
8.0 10^4
0 5 10 15 20 25 30 35
FUSION PROTEIN CONC. (fmol/ml)

FIG-7B

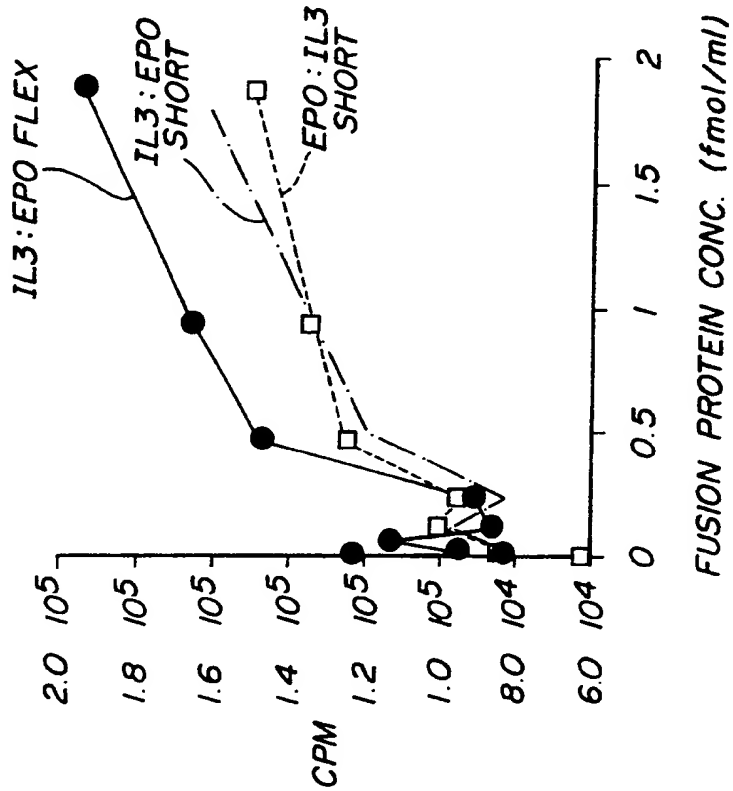

IL3:EPO FLEX
IL3:EPO SHORT
EPO:IL3 SHORT
CPM
2.0 10^5
1.8 10^5
1.6 10^5
1.4 10^5
1.2 10^5
1.0 10^5
8.0 10^4
6.0 10^4
0 0.5 1 1.5 2
FUSION PROTEIN CONC. (fmol/ml)

FIG-8B

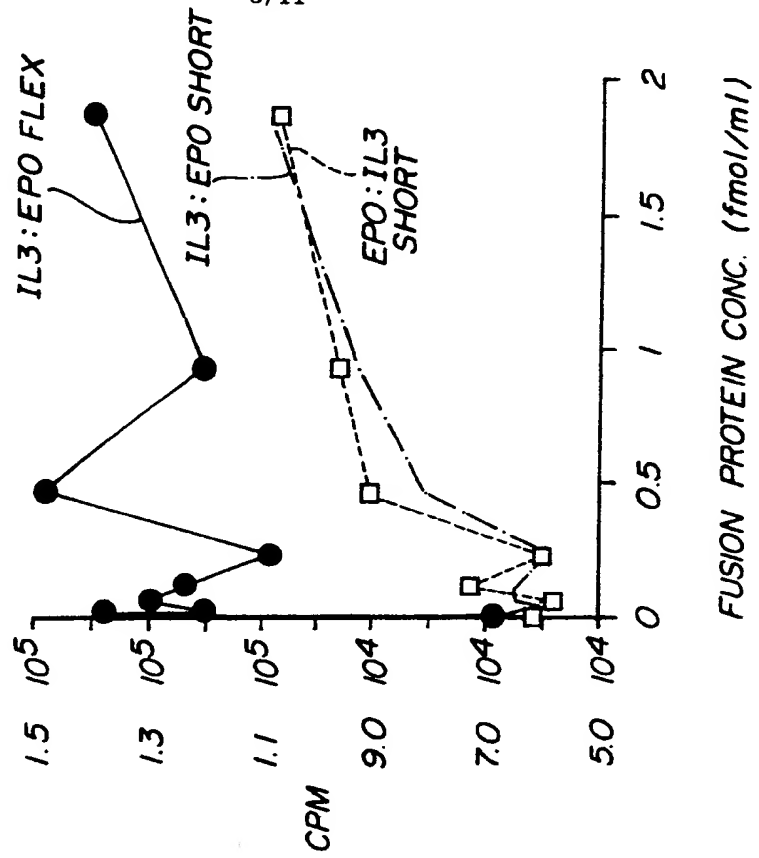

IL3:EPO FLEX
IL3:EPO SHORT
EPO:IL3 SHORT
CPM
1.5 10^5
1.3 10^5
1.1 10^5
9.0 10^4
7.0 10^4
5.0 10^4
0 0.5 1 1.5 2
FUSION PROTEIN CONC. (fmol/ml)

FIG-8A

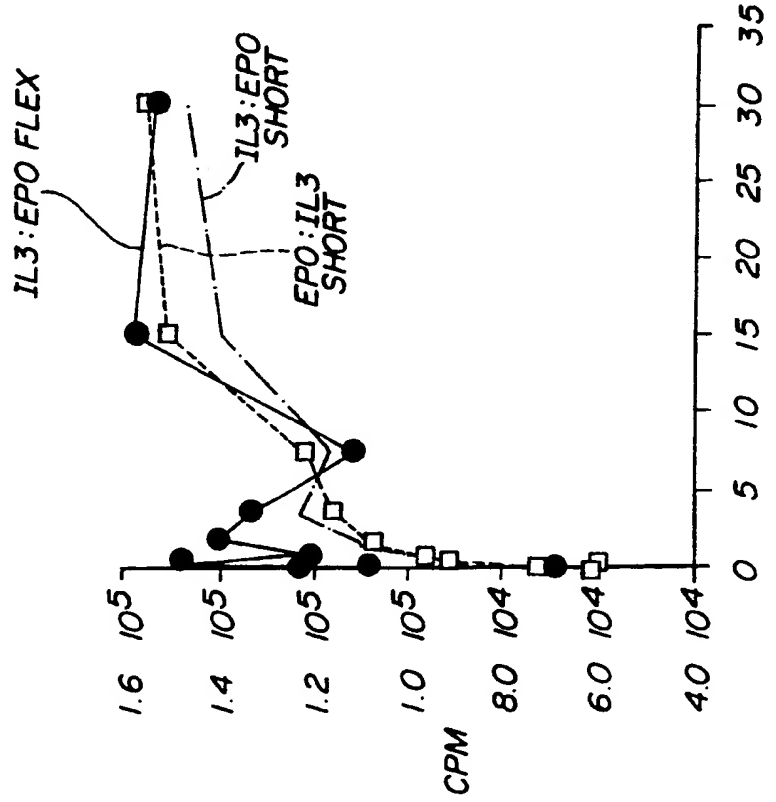

IL3:EPO FLEX
IL3:EPO SHORT
EPO:IL3 SHORT
CPM
1.6 10^5
1.4 10^5
1.2 10^5
1.0 10^5
8.0 10^4
6.0 10^4
4.0 10^4
0 5 10 15 20 25 30 35
FUSION PROTEIN CONC. (fmol/ml)

FIG-9

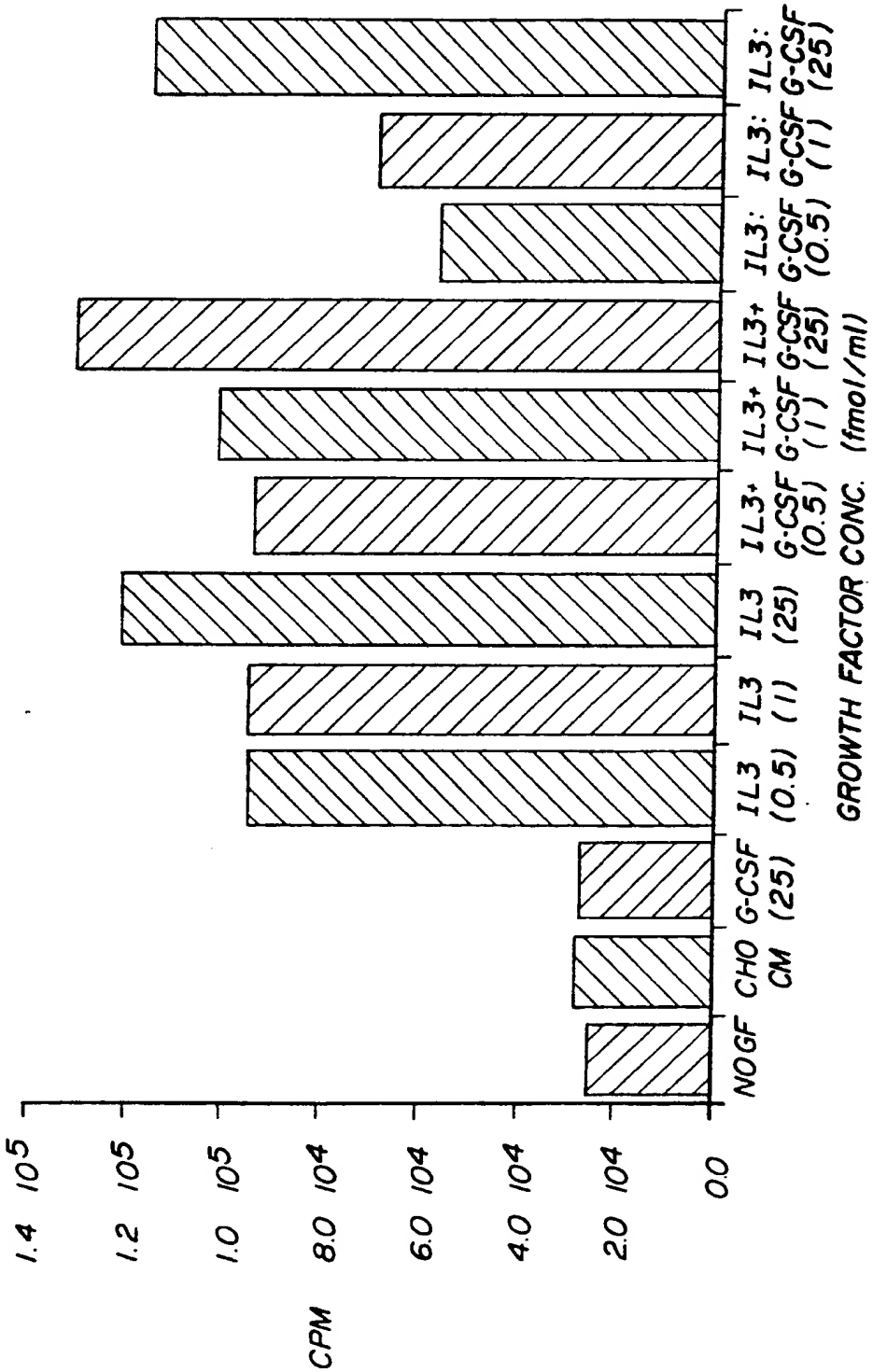

CPM
1.4 10^5
1.2 10^5
1.0 10^5
8.0 10^4
6.0 10^4
4.0 10^4
2.0 10^4
0.0
NO GF
CHO CM
G-CSF (25)
IL3 (0.5)
IL3 (1)
IL3 (25)
IL3+ G-CSF (0.5)
IL3+ G-CSF (1)
IL3+ G-CSF (25)
IL3: G-CSF (0.5)
IL3: G-CSF (1)
IL3: G-CSF (25)
GROWTH FACTOR CONC. (fmol/ml)

FIG-10

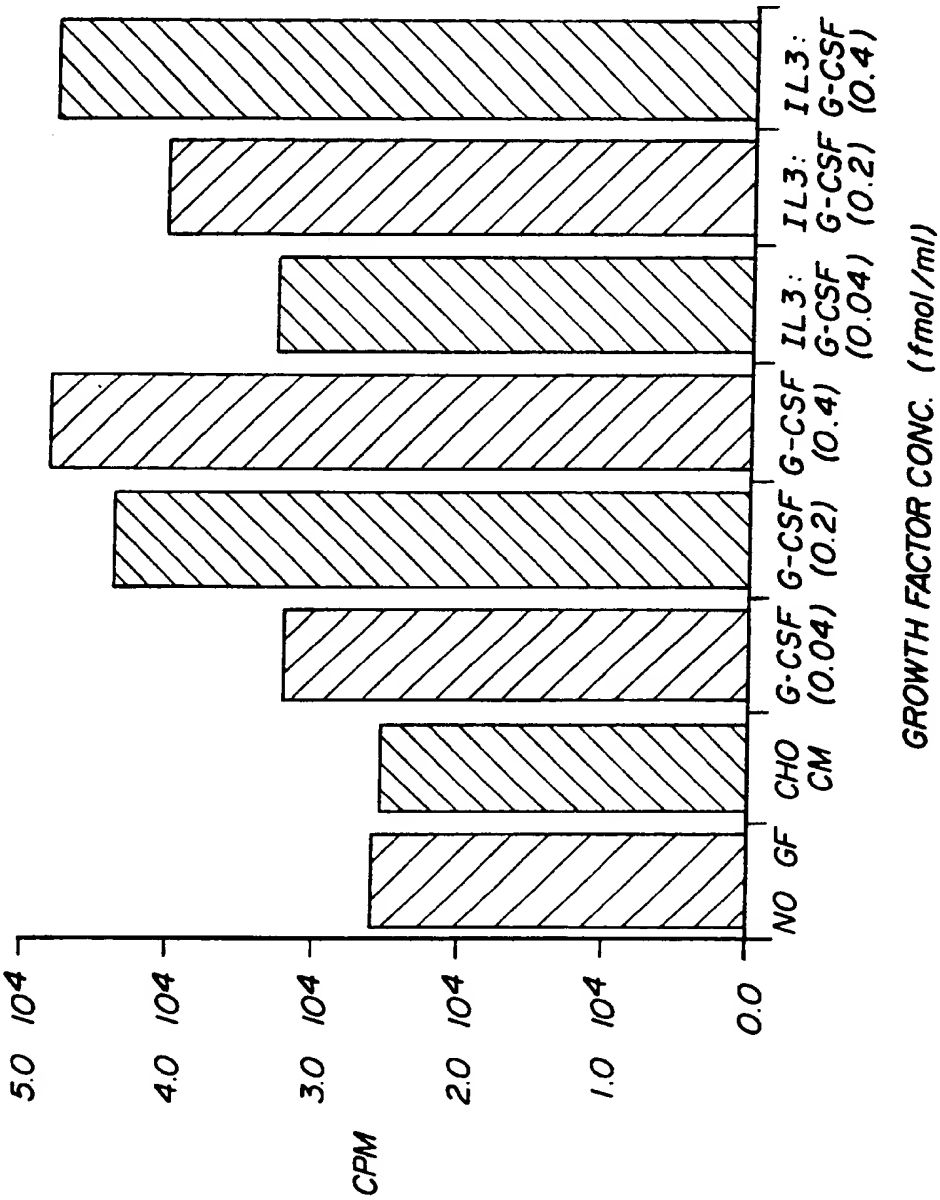
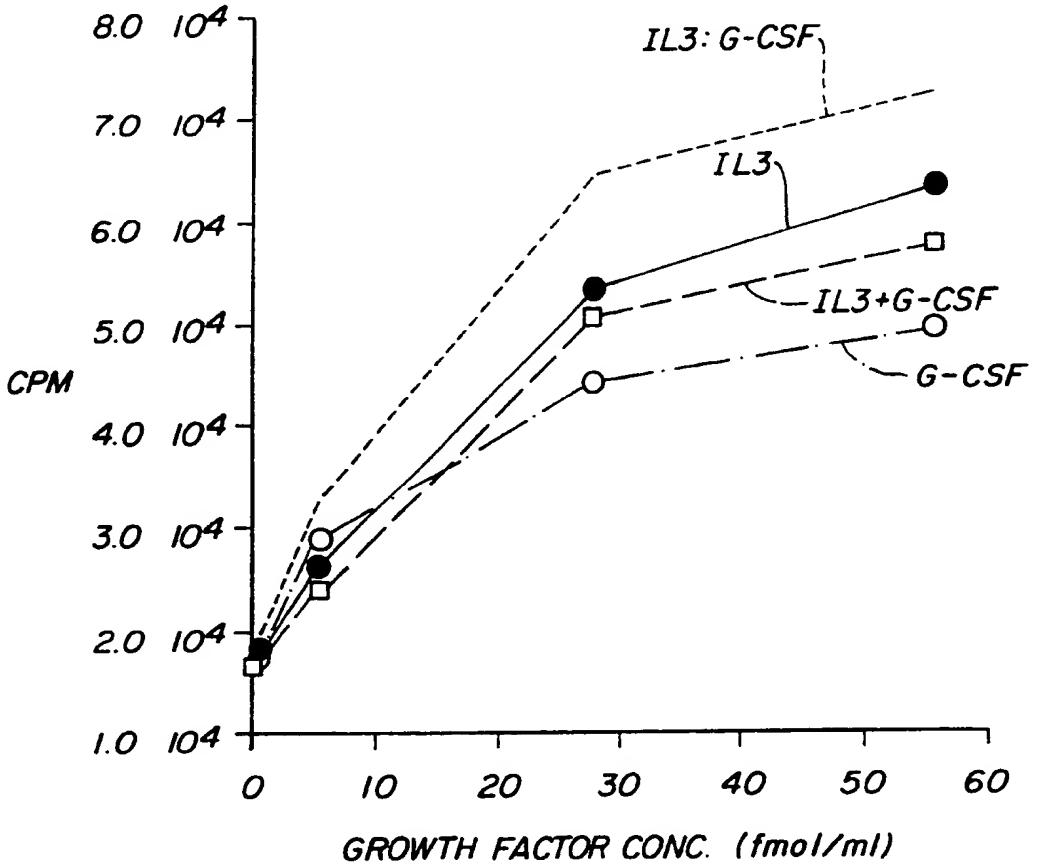

5.0 104
4.0 104
3.0 104
2.0 104
1.0 104
0.0
CPM
NO GF
CHO CM
G-CSF (0.04)
G-CSF (0.2)
G-CSF (0.4)
IL3: G-CSF (0.04)
IL3: G-CSF (0.2)
IL3: G-CSF (0.4)
GROWTH FACTOR CONC. (fmol/ml)

FIG-11


8.0 10⁴
7.0 10⁴
6.0 10⁴
5.0 10⁴
4.0 10⁴
3.0 10⁴
2.0 10⁴
1.0 10⁴
CPM
IL3: G-CSF
IL3
IL3+G-CSF
G-CSF
0
10
20
30
40
50
60
GROWTH FACTOR CONC. (fmol/ml)

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/07053

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5):C07K 15/00; C12P21/02; C12N 15/24, 15/27, 15/70, 5/00,5/10; A61K 37/02; C07H 15/12

U.S .CL.: 530/351,395;435/69.5,69.51,69.6,69.7, 172.3,240.1,320.1;424/85.1; 536/27

II FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	530/350,351,395; 435/69.1,69.5,69.51, 69.52, 69.6,69.7,172.3 240.1,252.3, 320.1; 424/85.1,85.2; 536/27

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

Computer Data-base search

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
E, X Y	US. A. 5.073.627 (Curtis et al.) 17 December 1991. see claims.	1-2.20-26,4 1-26
Y	US. A. 4.935.233 (Bell et al.) 19 June 1990. see col. 3-4 and claims.	1-26
Y	US. A. 4.935.352 (Koichi et al.) 19 June 1990. see all.	1-26
P.Y	WO. A. 91/01004 (Svrluga et al.) 24 January 1991. see all.	1-26
A	US. A. 4.675.382 (Murphy) 23 June 1987. see all.	1-26
Y	WO. A, 88/00971 (Ramshaw et al.) 11 February 1988. see all.	1-26

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
09 January 1992	07 FEB 1992

International Searching Authority	Signature of Authorized Officer
ISA/US	Garnette D. Draper ebw